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STUDIES OF THE METABOLISM OF ANDROGENS IN WOMEN[1,2]

E. C. HAMBLEN, R. A. ROSS, W. KENNETH CUYLER,[3]
MARGARET BAPTIST AND CATHERINE ASHLEY

From the Endocrine Division of the Department of Obstetrics and Gynecology, Duke University School of Medicine and Hospital
DURHAM, NORTH CAROLINA

KORENCHEVSKY has made these pertinent observations.

> It is most improbable that the presence of male compounds in the female organism is without significance or without effect on the sexual and other organs and functions . . . It follows that the endocrine treatment of diseases of women cannot be perfected until 1) the male sexual hormones present in females have been isolated and identified; 2) a thorough experimental investigation of the physiological and pathological properties of these hormones, alone, and in combination with oestrogenic hormones and progesterone, has been carried out; 3) the normal ratio of oestrogenic hormones to progesterone and to male hormones in normal women has been ascertained; 4) the pathological disturbance of this ratio during endocrine and gynecologic disturbances in women has been discovered.

A year ago our group began a comprehensive study of the gynecic rôle of androgens in health and in disease. This paper presents some of the varied data which are being secured. Subsequent reports will be concerned with various aspects of the problem.

According to Callow (2), androgens were identified first in the urines of males by Loewe and Voss in 1929. Womack and Koch (3) in 1932 reported that normal women excreted quantities of androgens in the urine comparable to those excreted by normal males. Butenandt and his coworkers (4) identified the androgens in the urines of males as androsterone and transdehydroandrosterone. Callow and Callow (5) isolated these same two androgens from the urine of normal women and, thereby, established that no apparent chemical difference exists between the androgens excreted by the two sexes. David (6) in 1935 recovered testosterone from bull testes. The existence of androgens in human testes or ovaries has not been established.

Studies of the androgenic titers of repeated 24-hour specimens of urine from large series of subjects have been impossible until recently due to the limitations imposed by the necessity of identifying androgens by bioassay. Koch (7), from determinations by bioassay upon 'pooled' 2 to 4-day specimens of urine, secured over

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[3] Ciba Fellow in Clinical Endocrinology.

periods of 27 to 44 consecutive days, has given the average range of excretion of androgens for men (aged 25 to 35 years) as 40 to 100 I.U. and, for women (aged 23 to 34 years), 30 to 100 I.U. Striking variations were encountered in the values for different 'pooled' specimens of the same individual. More marked variations were anticipated when 24-hour specimens were used.

The excretion of androgens is said to be greatest during the period of sexual activity. Androgenic titers are low in boys and girls and increase as adolescence occurs (7–14). There is a paucity of reports concerning androgenic titers after the climacteric and in senility. Dingemanse and associates state that these values are low in aged subjects of both sexes.

No characteristic fluctuations in androgenic titers during the menstrual cycle have been established. Koch (7) has observed that women tend to excrete less androgens during menstruation. He encountered no characteristic alterations in androgenic titers in association with fluctuations of urinary estrogens during the cycle. Dingemanse and his associates (10) reported from studies upon a single patient that the greatest excretion of androgens occurred during the postmenstrual phase of the cycle. Callow (2) has observed a similar tendency.

There have been conflicting reports concerning the effects of gonadectomy upon androgenic titers. McCullagh and Renshaw (15) found no androgenic activity in the urine of 11 eunuchs. Koch (16) found androgens present but in small amounts. Kenyon and associates (17) made similar observations. Chou and Wu (12), however, found definite androgenic activity in the urine of a eunuch 68 years of age. Callow (2) found, on the other hand, that eunuchoids had urinary values for androgens equal to or somewhat higher than those of normal men while true eunuchs showed a low level of androgenic excretion, which, however, was not outside normal limits. Callow reported likewise, that oöphorectomized women excreted androgens but in somewhat smaller amounts than most normal women.

Because of the continued excretion of androgens by patients after orchidectomy or oöphorectomy and because of the decreased excretion of androgens by patients with Addison's disease and the increased excretion by patients with adrenogenitalism, Callow suggested that the chief source of these androgenic principles was the adrenal cortex.

Reports upon the effects of injections of certain androgens, particularly testosterone propionate, have dealt almost exclusively with male patients, for the most part eunuchs or hypogonadal individuals. These reports have shown considerable disagreement. Kochakian (9) observed no significant alterations in the urinary titers of a normal male following injections of 11 to 17 capon units of androgens on two occasions. He found that daily injections of 30 to 40 capon units for 10 days to a castrated male resulted in the recovery of approximately 3% of the androgen administered. However, Callow (2) found no definite changes in the titers of 15 out of 17 men with prostatic hypertrophy who were receiving 10 to 100 mg. of testosterone propionate weekly. Two patients, who received 50 to 100 mg. respectively, excreted somewhat larger amounts of androgens, their titers rising from 15 to 50 I.U. McCullagh, Rumsey and Cuyler (18) reported that injections of 5 to 10 mg. of testosterone propionate in patients with hypogonadism caused some rise in androgenic titers but did not raise these to normal levels. Larger doses increased these titers to normal ones. They estimated that between 20 and 70% of the injected androgen could be recovered in the urine. Dorfman and Hamilton (19) reported upon numerous bioassays of the urine of a 35-year-old man with one intra-abdominal testis who was

treated intramuscularly with daily doses of 20 mg. of testosterone propionate for 30 days. The average daily excretion before treatment was 13 I.U. The titers during therapy varied between 47 and 97 I.U. Marked increases in the daily urinary titers, these reaching 500 I.U., occurred in the same patient following the oral administration daily of 60 mg. of testosterone propionate. Hoskins, Coffman, Koch and Kenyon (20) reported that the intramuscular injection of 25 mg. of testosterone propionate per day for 9 to 14 days to 4 eunuchoids restored both the androgenic and estrogenic titers to normal levels: androgenic titers were raised from 2 to 16 I.U. per day to 44 to 115 I.U. per day and estrogenic titers from the equivalents of 0.3 to 5 γ of estrone per day to those of 5 to 15 γ of estrone per day. Values returned to pre-treatment levels when the injections were discontinued. They suggested that these findings might indicate that the urinary estrogens of the male might be derived in part from the metabolism of androgens.

No studies have been encountered which dealt with the effects of administrations of testosterone propionate upon the hormonal titers of the female. Significant information, especially in regard to alterations in the metabolism of estrogens and progesterone, seems highly desirable at this time since there has been begun during the past year a rather general use of androgens in the treatment of most functional conditions in women, for which estrogens have been reported to have been of value. Hamilton and Dorfman (21) have called attention to the fact that in the male possible damage may follow the use of large amounts of the hormone. They emphasize the widespread effects which have been observed following therapy: cardiac enlargement, alterations in pulse rate and blood pressure, and changes in muscles, skin (22) and vision. Reports have appeared already regarding the production of masculinization (23) and enlargement of the clitoris in women (24) who had been treated with androgens.

METHODS

Wu and Chou (25), Oesting (26), Callow and Emmens (27) and others have described colorimetric methods for the quantitative estimation of androgens in extracts of urine. These methods are based upon a color reaction, Zimmermann reaction (28), of the sex sterols with meta-dinitro-benzene in alkaline solution.

We have employed Oesting's method with the exception of two essential modifications, devised by one of us (W.K.C., 29): (*a*) In the preliminary extraction dibutyl ether has been employed instead of benzol because of the definite advantages it possesses as a solvent for sex sterols, it is practically non-toxic and non-inflammable; few urochromes are soluble in it; and it requires simpler apparatus. (*b*) The dibutyl-ether extract is washed prior to distillation with weak and strong alkalis to remove the estrogens. The technic of colorimetric determinations employing the Oesting-Hellige colorimeter has been unaltered. All colorimetric readings have been made by one of us (M.B.) in order to minimize personal errors.

Consecutive 24-hour specimens of urine have been titrated. Colorimetric quantitation has been done promptly after hydrolysis and extraction of the specimens. Results are reported as international units of androsterone for the 24-hour specimen. These data permit no inference as to the biologic activity or as to the chemical nature of the androgens quantitated.

PRESENTATION AND ANALYSIS OF DATA

Healthy Women Having Normal Menstrual Cycles

The urinary titers of androgens and sodium pregnandiol glucuronide during one or more menstrual cycles of 5 white women whose ages varied from 21 to 43 years are presented in figure 1. The oldest patient (43 years)

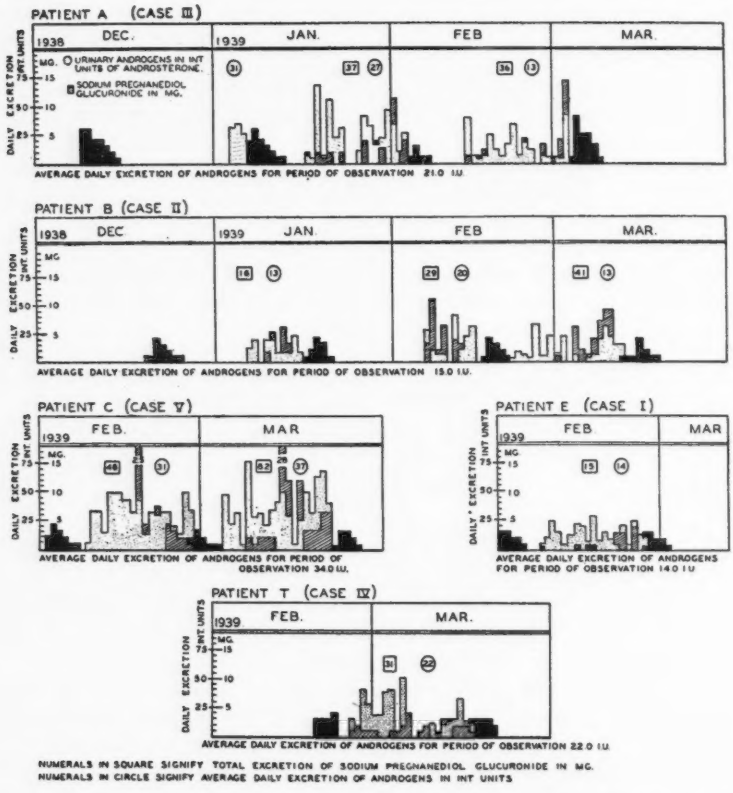

PATIENT A (CASE III)
1938 DEC.
1939 JAN.
FEB
MAR.
DAILY EXCRETION INT UNITS
MG. URINARY ANDROGENS IN INT UNITS OF ANDROSTERONE.
SODIUM PREGNANEDIOL GLUCURONIDE IN MG.
AVERAGE DAILY EXCRETION OF ANDROGENS FOR PERIOD OF OBSERVATION 21.0 I.U.
PATIENT B (CASE II)
AVERAGE DAILY EXCRETION OF ANDROGENS FOR PERIOD OF OBSERVATION 15.0 I.U.
PATIENT C (CASE V)
AVERAGE DAILY EXCRETION OF ANDROGENS FOR PERIOD OF OBSERVATION 34.0 I.U.
PATIENT E (CASE I)
AVERAGE DAILY EXCRETION OF ANDROGENS FOR PERIOD OF OBSERVATION 14.0 I.U.
PATIENT T (CASE IV)
AVERAGE DAILY EXCRETION OF ANDROGENS FOR PERIOD OF OBSERVATION 22.0 I.U.
NUMERALS IN SQUARE SIGNIFY TOTAL EXCRETION OF SODIUM PREGNANEDIOL GLUCURONIDE IN MG.
NUMERALS IN CIRCLE SIGNIFY AVERAGE DAILY EXCRETION OF ANDROGENS IN INT UNITS

Fig. 1. Urinary androgens of 5 healthy women who had cyclic menses. Twenty-four-hour specimens of urine were titrated. Episodes of menstruation are shown by solid black blocks. Daily androgenic titers are denoted quantitatively as I.U. of androsterone by the stippled blocks. Daily urinary titers of sodium pregnandiol glucuronide in mg. are indicated by the obliquely hatched blocks. Average daily titers of androgens for a cycle are inserted in circles. Total cyclic excretions of sodium pregnandiol glucuronide are inserted in squares. The average daily excretion of androgens for the entire period of observation is presented at the bottom of each menstrual chart. Patient *T* (*Case IV*) had endometrial biopsies at the onset of each episode of bleeding shown. These biopsies showed normal menstrual endometrial patterns.

was verified to have experienced normal menstruation, by endometrial biopsies taken at the onset of several periods. In all 143 24-hour specimens of urine were titrated for androgens.

Correlation of the 'coloring' and 'complexion' of these women with their average daily excretion of androgens yielded the following data:

Case I, Patient E, blonde, 14 I.U.
Case II, Patient B, medium blonde, 15 I.U.
Case III, Patient A, medium brunette, 21 I.U.
Case IV, Patient T, medium brunette, 22 I.U.
Case V, Patient C, marked brunette, 34 I.U.

Immediate Effects of Gynecologic Surgery

(*a*) *Hysterectomy with ovarian conservation.* The effects of this type of operative procedure were investigated in 2 patients. In all, 25 24-hour specimens of urine were titrated for androgens. *Case VI* was a colored woman, aged 32 years, who had had a left salpino-oöphorectomy and supravaginal hysterectomy. *Case VII* was a colored woman, aged 25 years, who had had supravaginal hysteromyomectomy, bilateral salpingectomy, right oöphorectomy and partial left oöphorectomy. The data secured upon these patients are presented in table 1.

TABLE 1. ANDROGENIC TITERS AFTER HYSTERECTOMY

	Case VI	*Case VII*
	I.U.	I.U.
Daily androgenic titers	6	7
	6	
Before operation[1]	16	
	20	4
	50	24
	144	25
	72	6
	15	27
After operation	50	27
	14	27
	14	3
	14	45
	43	10
		16
Average daily titers	36	18

[1] Longer preoperative studies were impossible because of operative schedules.

(*b*) *Hysterectomy and bilateral oöphorectomy.* The immediate effects of these operations were investigated in 5 patients. In all 79 24-hour specimens of urine were titrated for androgens. *Cases VIII, IX, X, XI* and *XII*, were 5 colored women, whose ages were respectively 30, 35, 37, 41, and 44 years and who all had had operations involving hysteromyomectomy and bilateral salpingo-oöphorectomy. The data secured upon these patients are presented in table 2.

The Effects of Functional Alterations in the Endocrine System

(*a*) *Delayed adolescence and menarche due to hypoovarianism.* The androgenic titers were investigated in 2 white girls who had delayed menarche and retarded adolescent development. In all, 70 24-hour specimens of urine were titrated for androgens.

Case XIII was a white girl, aged 14½ years, who had had retarded statural growth and sexual maturation. The patient was of blonde coloring. Menarche had not occurred. Diagnosis was that of hypoantuitarism.

Case XIV was a white girl, aged 19 years, who had had retarded statural growth

TABLE 2. ANDROGENIC TITERS AFTER HYSTERECTOMY AND OÖPHORECTOMY

	Case VIII	*Case IX*	*Case X*	*Case XI*	*Case XII*
	I.U.	I.U.	I.U.	I.U.	I.U.
Daily androgen titers Before operation[1]	7	7	21	14	16
	11	5			4
	12	13			52
After operation	13	10	13	30	4
	9	7	22	9	35
	25	18	12	6	28
	16	10	54	41	8
	12	11	25	19	61
	11	5	4	58	54
	5	5	36	16	3
	10	11	7	45	37
	22	7	4	36	8
		7	16	32	19
		4	6	42	20
			12	32	36
					7
					47
					35
					3
					24
					24
					32
					23
					22
					17
					19
					32
Average daily titers	13	9	18	29	21

[1] Longer preoperative studies were impossible because of operative schedules.

and incomplete sexual maturation. The patient was a marked brunette. Menarche had not occurred. She had had intensive therapy with pituitary fractions and thyroid substance during the 2½ years prior to the present period of observation. During the interval reported 3 episodes of bleeding had been induced by therapy with estrogens and progesterone. Endometrial biopsies, secured at the onset of these episodes of bleeding, showed no progestational alterations in the endometria. Diagnosis was that of Turner's syndrome. The data secured upon these patients are presented in table 3.

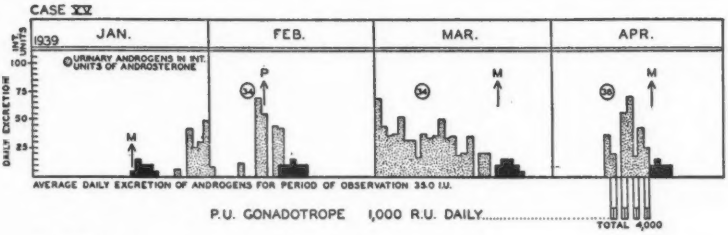
(*b*) *Menometrorrhagia.* The androgenic titers were investigated in 2 patients who had had menometrorrhagia. In all, 88 24-hour specimens of urine were titrated for androgens.

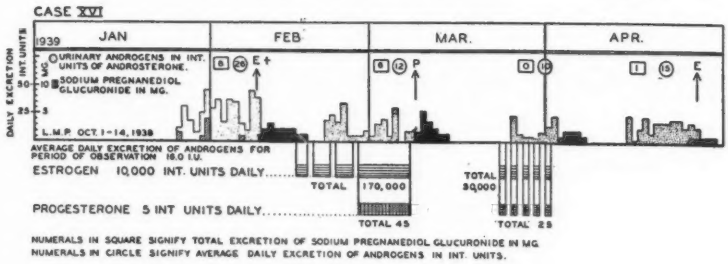
Case XV was a colored girl, aged 17 years, who had had episodes of severe metrorrhagia for 2 years prior to initiation of therapy. The patient had had 5 consecutive series of cyclic therapy with the sex sterols and 3 cycles of no therapy, prior to the interval covered by the menstrual and therapy charts in figure 2. The endometrium at the beginning of therapy was of hyperestrogenic character; it had changed to one of mixed character under therapy. Bleeding had been of cyclic type and normal in amount and duration during therapy. The androgenic titers and endometrial findings during the succeeding 3 cycles are shown in figure 2.

Case XVI was a white woman, aged 29 years, who had had episodes of amenorrhea and of severe metrorrhagia since menarche at 15 years. Androgenic titers and endometrial findings during 4 cycles are shown in figure 3.

(c) *Amenorrhea.* The androgenic titers were investigated in 4 white women having episodes of amenorrhea which have varied in duration from 4 months to 5 years. In all, 127 24-hour specimens of urine were titrated for androgens.

Case XVII was a white woman, aged 26 years, who had had episodes of amenorrhea varying between 4 and 12 months, since menarche at 13 years of age. She was of





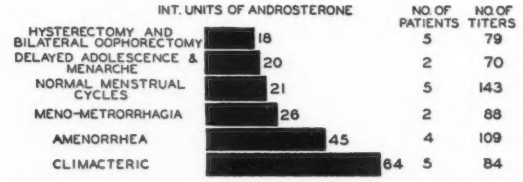


Fig. 2. (Upper) The urinary androgens of the patient described as *Case XV*. The menometrorrhagia of this patient had been controlled by 5 previous cycles of therapy although moderate irregularity of progestational differentiation persisted in her endometria. The same conventions in graphing which are described in legend of figure 1 are employed here. Endometrial biopsies are indicated by ↑. The letter M indicates a mixed endometrium. The letter P indicates a normal progestational endometrium.

Fig. 3. (Center) The urinary androgens of the patient described as *Case XVI*. The same conventions in graphing which are described in legend of figure 1 are employed here. The letter P indicates a normal progestational endometrium. The letter E indicates a persistent estrogenic endometrium. The symbol E+ indicates a hyperestrogenic endometrium. Endometrial biopsies are indicated by ↑.

Fig. 4. (Lower) Composite data in regard to androgenic titers of different groups of patients investigated. The titers reported represent the average daily excretion for all the members of a single group.

blonde complexion with a generalized unpigmented hirsutism. The endocrine diagnosis was primary hypoovarianism. The episode of amenorrhea investigated had lasted 4 months.

Case XVIII was a white girl, aged 16 years, who had had amenorrhea of 12 months duration associated with diabetes mellitus. She was of marked brunette coloring. The endocrine diagnosis was secondary hypoovarianism due to diabetes mellitus.

Case XIX was a white woman, aged 30 years, para 4-1-3, who had had amenor-

rhea of 14 months duration. She was of moderate marked brunette coloring. The endocrine diagnosis was secondary hypoovarianism.

Case XX was a white woman, aged 26 years, who had had oligomenorrhea since menarche at 14 years of age and amenorrhea of 5 years duration. She was of moderate brunette coloring. The endocrine diagnosis was primary hypo-ovarianism. The data secured upon these patients are presented in table 4.

TABLE 3. ANDROGENIC TITERS OF PATIENTS WITH DELAYED MENARCHE

	Case XIII	*Case XIV*
	I.U.	I.U.
Daily androgenic titers	12	25
	8	26
	6	20
	8	12
	16	28
	10	8
	4	5
	4	12
	14	32
	18	4
	2	4
	12	60
	8	44
	12	48
	5	29
	4	47
	5	12
	17	29
	17	7
	43	32
	14	36
	42	57
	17	18
	20	36
	28	18
	18	13
	13	36
	23	17
	22	19
		46
		11
		40
		31
		31
		37
		23
		23
		20
		31
		23
		14
Average androgenic titers	15	25

(d) *Climacteric.* The androgenic titers were investigated in 5 post-menopausal white women. The intervals elapsing since the last menstrual period varied from 9 months to 26 years. A total of 84 24-hour specimens of urine was titrated for androgens.

Case XXI was a white woman, aged 42 years, who had had the last menstrual period 9 months prior to initiation of studies. Diagnosis was that of spontaneous menopause.

Case XXII was a white woman, aged 48 years, who had had the last menstrual period 2 years prior to the initiation of studies. Diagnosis was spontaneous menopause.

Case XXIII was a white woman, aged 62 years, who had had the last menstrual period 14 years prior to the initiation of studies. Diagnosis was spontaneous menopause.

TABLE 4. ANDROGENIC TITERS IN AMENORRHEA

	Case XVII	*Case XVIII*	*Case XIX*	*Case XIX*	*Case XX*
Daily androgenic titers	I.U. 70 75 17 29 77 19 46 29 14 31 34 31 50 31 10 7 29 10 31 43 24 34 7 17 5 29 7 39 10 17 17 22 31 43 19 17 22 31 19 17 26	I.U. 29 19 2 6 4 27 23 11 108 7 5 63 34 7 54 126 78 13 41 102 97 102 102 117 107 63	I.U. 67 46 26 57 26 36 22 34 48 79 29 67 34 79 72 117 94 79 77 119 148 65 76 72 101 101 76 72	(during the daily administration of 10,000 I.U. of estrone) I.U. 30 119 36 18 68 47 25 54 29 32 14 25 50 65 43 47 14 108	I.U. 13 34 43 36 34 47 14 14 38 38 23 40 34 54
Average daily titers	28	52	69	46	33

Case XXIV was a white woman, aged 48 years, who had had the last menstrual period 16 years prior to the initiation of studies. Diagnosis was that of surgical menopause (hysterectomy and oöphorectomy).

Case XXV was a white woman, aged 74 years, who had had the last menstrual period 26 years prior to initiation of studies. Diagnosis was spontaneous menopause. The data secured upon these patients are presented in table 5.

The Effects of Injections of Testosterone Propionate

The androgenic titers were investigated in 4 white women before and during the intramuscular injections of various amounts of testosterone propionate.[4] In all, 89 24-hour specimens of urine were titrated for androgens. (Since 33 of these determinations have been reported in consideration of the climacteric as the data of *Case XXII* and *XXIV*, actually only 56 additional determinations are reported).

Case XXIV was a white woman, aged 48 years, who had had the last menstrual period 16 years prior to the initiation of the studies (pretreatment record reported under climacteric). Diagnosis was surgical menopause (hysterectomy and oöphorectomy). During the treatment period, intramuscular injections of 10 mg. of testo-

TABLE 5. ANDROGENIC TITERS IN THE CLIMACTERIC

	Case XXI	*Case XXII*	*Case XXIII*	*Case XXIV*	*Case XXV*
	I.U.	I.U.	I.U.	I.U.	I.U.
Daily androgenic titers	25	112	80	13	36
	22	60	49	11	34
	83	108	49	18	40
	104	63	54	9	27
	94	162	65	54	12
	22	119	9	52	40
	65	86	80	43	45
	112		13	18	39
	101		63	59	39
	180		76	9	36
	94		70	14	36
			76	77	43
			25	36	36
			76	54	45
			43	23	45
			38	23	19
				23	39
				47	27
				23	45
				36	26
				54	47
				48	42
				54	28
				47	27
				25	
				49	
Average daily titers	91	101	60	35	36

sterone propionate were given every 2 or 3 days for total dosage of 80 mg. Determinations were carried out on 3 consecutive 24-hour specimens of urine after the discontinuation of therapy.

Case XXII was a white woman, aged 48 years, who had had the last menstrual period 2 years prior to the initiation of studies (pretreatment record reported under climacteric). Diagnosis was spontaneous menopause. During the treatment period, intramuscular injections of 25 mg. of testosterone propionate were given daily for the first 9 days for a total dosage of 225 mg. Determinations were carried out on 3 consecutive 24-hour specimens of urine after the discontinuation of therapy.

Case XXVI was a white woman, aged 67 years, who had had the last menstrual period 25 years prior to initiation of studies. Diagnosis was spontaneous menopause. During the treatment period, intramuscular injections of 50 mg. of testosterone

[4] Perandren (Ciba Pharmaceutical Products Co., Summit, N. J.)

TABLE 6. ANDROGENIC TITERS AFTER INJECTIONS OF TESTOSTERONE PROPIONATE

		Case XXIV	*Case XXII*	*Case XXVI*	*Case XXVII*
Before therapy	Daily androgenic titers	(Reported under climacteric)		I.U. 16 19 47 22	I.U. 11 25 27 54 34 52 32
	Average daily titer	I.U. 35	I.U. 101	26	34
	Daily androgenic titers	34 59 17 29 26 49 87 70 68 86 68 86 130 67 76 76 97 45 41 59	120 252 74 137 133 115 137 88 101 74 81	90 288 225 135 135 42 63 63 63 60 18 32	81 63
During therapy	Average daily titer	64 I.U.	119 I.U.	101 I.U.	72 I.U.
	Total mg. testosterone propionate given	80 mg.	225 mg.	500 mg.	100 mg.
	Total increase in androgenic titers attributable to therapy	570 I.U.	201 I.U.	902 I.U.	76 I.U.
	Theoretical increase (1 mg. 10 I.U.)	800 I.U.	2250 I.U.	5000 I.U.	1000 I.U.
	Percentage of theoretical yield recovered	71%	9%	18%	7.6%

propionate were given daily for 10 days for a total dosage of 500 mg. Determinations were carried out on 3 consecutive 24-hour specimens of urine after the discontinuation of therapy.

Case XXVII was a white woman, aged 33 years, who had had supravaginal hysteromyomectomy and bilateral salpingo-oöphorectomy 2 days prior to the initiation of studies. During the treatment period, a single intramuscular injection of 100 mg. of testosterone propionate was given. Determinations were carried out upon 2 consecutive 24-hour specimens following the injection. The data secured upon these patients are presented in table 6.

DISCUSSION

The colorimetric method employed by us has been established as comparing favorably with other methods for quantitation of androgens by Oesting

and Webster (13) and by Nathanson, Towne and Aub (14). Both of these groups investigated the androgenic titers of children. Oesting and Webster employed both the colorimetric method and that of bioassay. They found results in good agreement.

The colorimetric method yields only data in regard to amounts of total androgens present. No inferences are permitted as to the chemical forms or the pharmacologic activity of the sterols titrated. Consequently, qualitative alterations in the androgens by metabolism or in altered functional conditions cannot be recognized. Investigations of these must await refinement in the technic of differential quantitation.

The data reported in this communication comprised 672 estimations upon 24-hour specimens obtained from 27 female patients. All workers, despite the technic employed in quantitative studies of androgens, have emphasized the occurrence of marked variation in the titers of different urinary specimens from the same patient. This fact makes the investigation of repeated specimens necessary in order that significant average daily values may be obtained. Single determinations cannot be accepted as yielding authentic data.

No characteristic cyclic fluctuations in androgenic titers similar to those described for estrogens and for progesterone (pregnandiol complex) were encountered in our studies of 9½ menstrual cycles which occurred in 5 healthy women. The values obtained for the pregnandiol complex in all 5 of these patients as well as their histories, physical characteristics and the regularity of their menstrual cycles leave little doubt that they constituted an excellent group of normal subjects. One of these patients (*Case IV*) who was 43 years of age was studied by endometrial biopsies at the onset of the menstrual flowings which preceded and succeeded the interval between which titers were estimated. Both endometrial specimens were of normal menstrual character.

The correlation established between the 'complexion' or 'coloring' of these normal women and their average daily androgenic titers has been described in a preliminary report by two of us (E.C.H. and W.K.C., 30). A similar correlation seemed apparent in other patients in this group who could not be classed as distinctly normal and whose titers were influenced by other factors being investigated. These correlations seemed significant in view of the apparent 'developing' action of testosterone, when injected intramuscularly, upon melanin of patients previously exposed to sunlight or ultra-violet rays, as reported by Hamilton and Hubert (23). There seems to be some relationship between androgenic titers and tolerance for exposure to actinic rays in the group of patients investigated by us.

Since no estimations of androgens were carried out by our group during periods of menstrual flowing or uterine bleeding, no data are available in regard to the previously reported observations that androgenic titers tend to be lower at the time of menstrual bleeding.

Neither hysterectomy with conservation of the ovarian tissue or hysterectomy with bilateral oöphorectomy has any apparent immediate effect upon

the urinary titers of androgens. This apparent noneffect of hysterectomy contrasts with the observations reported by our group (31) upon the excretion of sodium pregnandiol glucuronide. Hysterectomy or curettage during the progestational phase of the menstrual cycle was found to be followed by a prompt disappearance of the pregnandiol complex from the urine. According to Smith and Smith (32), the endometrium is also necessary for the complete metabolism of the various estrogens. These studies of our group, however, do not rule out the possibility that qualitative alterations may occur in androgenic titers following these operative procedures.

The fact that no significant quantitative alteration occurs in the androgenic titers following complete oöphorectomy indicates that the ovaries are not the primary source of the urinary androgens. The intact ovary, however, may induce qualitative alterations in the metabolism and the utilization of the androgens supplied to that glandular structure. There is some evidence (*Case VIII*, *IX* and *X*) that the average daily androgenic titers may be somewhat lowered immediately following oöphorectomy. This would seem to be more apparent in the younger patients than in those approaching the climacteric. Individual daily values, however, in those patients having somewhat depressed average daily values, may be relatively high. Callow (2) has observed also some moderate depression in urinary titers following oöphorectomy. The discussion which follows in a consideration of climacteric patients suggests that androgenic titers rise as a late effect of oöphorectomy.

Early hypoovarianism, either primary or secondary, was investigated. Two young patients, aged 14½ and 19 years, who showed marked somatic and sexual retardation, characterized by delayed menarche, excreted amounts of androgens well within normal limits for their chronologic age. The undeniable hypofunction of the ovaries in these two patients did not result in any apparent lowering of the androgenic titers. These observations were not expected in view of the correlation established (14) between the degree of sexual development of children and their androgenic titers.

Menometrorrhagia in younger women, regardless of the degree and proliferation of the endometria associated with episodes of excessive bleeding, did not apparently associate itself with any uniform alteration in the average androgenic titers. One of these patients (*Case XVI*) did show higher titers prior to an episode of bleeding from a hyperestrogenic ('hyperplastic') endometrium than she did prior to bleeding from a normal menstrual or a persistent estrogenic endometrium. The average daily titers of androgens were 26 I.U. prior to the episode of bleeding from a hyperestrogenic endometrium. Cyclic therapy with sterols given according to the schema previously described by one of us (E.C.H., 33) resulted in a lowering of these average daily titers to approximately 13 I.U. The latter value according to our data is quite consistent with those of normal women of the same complexional characteristics. The effects of this therapy probably account for the alterations in titers prior to subsequent bleeding from menstrual and persistent estrogenic endometria. These two patients permitted observations to be made upon

these urinary titers of androgens prior to bleeding which occurred from persistent estrogenic, hyperestrogenic, mixed and menstrual endometria.

Episodes of amenorrhea, however, were apparently associated at times with alterations in the androgenic titers. These alterations were characterized for the most part by increases rather than decreases. Increased androgenic titers in association with adrenogenitalism and 'virilizing' syndromes have been reported (17, 34, 35). Many confirmatory studies of these reports have appeared. Our experience has not varied from these. We have not included in our report any women with 'virilizing' syndromes.

Androgenic titers were investigated in 4 young white women, whose ages ranged from 16 to 30 years and whose episodes of amenorrhea ranged in duration from 4 months to 5 years. The average daily androgenic titers of two of these patients (*Case XVIII, XIX*) whose amenorrhea had lasted 12 and 14 months respectively were considerably higher than those of the other two patients (*Case XVII, XX*) who had had no bleeding for 4 months and for 5 years respectively. These observations suggest that relatively short episodes of amenorrhea (those lasting only several months) probably are associated with no significant alterations in androgenic titers. Longer periods of amenorrhea, however, seem to result or to be associated with increase in these titers. Eventually, however, these increased titers become levelled to lower values if the amenorrhea continues for several years or more. The lowering of androgenic titers as amenorrhea progresses in its duration may indicate a scaling down of endocrine functions indicative of a more marked and more permanent ovarian failure.

The patients with amenorrhea investigated were those having either early or late hypoovarianism or having hypoovarianism secondary to diabetes mellitus. Hypopituitary and thyroid factors were not investigated.

The changes in androgenic titers in association with amenorrhea are quite similar to those observed in association with the climacteric. The average daily excretion of the entire group of patients with amenorrhea was 45 I.U. while similar data upon patients during and after the climacteric yielded an average value of 64 I.U.

The reports of Dingemanse and associates (10) led us to expect low titers in climacteric women. We found, however, that during the earlier months and years of the climacteric there was a definite increase in the androgenic titers. Three patients (*Case XXI, XXII, XXIII*) whose last episodes of bleeding had occurred 9 months, 2 years and 14 years previously showed definite elevations in androgenic titers as judged by values established for the other groups of patients. These 3 patients showed average daily values of 91 I.U., 101 I.U. and 60 I.U. respectively. The other 2 patients (*Case XXIV, XXV*) whose last episodes of bleeding had occurred 16 and 26 years previously excreted an average daily amount of 35 I.U. and 36 I.U. respectively.

These observations indicate the apparent occurrence of increases in androgenic titers as a part of the climacteric process. These increased androgenic titers apparently fall to lower levels as the climacteric progresses and

as somatic senescence advances. It is significant, however, that a patient 74 years of age excreted an average of 36 I.U. of androgens daily. The androgenic values therefore, do not appear to fall to subnormal levels even in senility.

When the composite average daily titers for all individuals of the various groups studied are compared, (fig. 4) there is a general and unmistakable grouping of the average titers into two classes: a low class and a relatively high class. The composition of these two classes is as follows.

Low Average Titers

Patients after hysterectomy and bilateral oöphorectomy—18 I.U.
Patients with delayed menarche—20 I.U.
Healthy women with normal cycles—21 I.U.
Patients with meno-metrorrhagia—26 I.U.
Patients after hysterectomy with ovarian conservation—27 I.U.

Relatively High Average Titers

Patients with amenorrhea—45 I.U.
Patients during climacteric and after climacteric—64 I.U.

These observations lead us to conclude, as did Callow (2), that the chief source of the total androgens which appear in the urine is not the ovary but most likely the adrenal. Primary hypoovarianism, intercurrent fluctuations in ovarian function, oöphorectomy and senility of the ovary do not result in subnormal androgenic titers.

The data secured in amenorrheic patients suggest that the determination of androgenic titers may permit the obtaining of significant information in regard to therapy and prognosis and may permit a more logical and effective therapeutic regime. There apparently are groups of patients in which androgenic titers rise during amenorrhea and others in whom these titers are not materially altered. In those patients who have increased androgenic titers it would seem logical to give estrogens therapeutically to reduce the androgenic titers as a preliminary step in therapy. The purpose of this preliminary treatment with estrogens would be to curtail any further defeminizing effects, such as regressional changes, which these androgens theoretically might produce. The subsequent treatment might well include additional estrogens to overcome these regressional effects and to prime the genital tract. The next step in therapy might logically be attempts at ovarian stimulation following the establishment of a more normal estrogen-androgen balance.

The apparent occurrence of increased titers of androgens during the early stages of the climacteric suggests a theory in extension of previous ones regarding the hormonology of the climacteric. These increased urinary titers of androgens may be regarded as resulting from a hyperactivity of the adrenal cortex. Other signs of hyperfunction of the adrenal cortex in the climacteric and in senescence are not wanting; these include hypertension, hirsutism, discoloration and pigmentation of the skin and others. The hyperactivity of the adrenal cortex may be related either directly to the hyperactivity of the anterior pituitary or to the absence of the regulatory effects

exerted by the ovaries upon the adrenal function. Both factors may be involved. Since androgens are known to depress the function of the pituitary when they are present in sufficient quantities and exert their action over sufficient periods of time, these may exert a conditioning effect upon the anterior pituitary similar to the effect previously exerted by active ovaries. Furthermore, the regressive alterations which occur after climacteric may be due to these increased androgenic titers rather than solely to the decrease in estrogenic titers. Clinically one encounters considerable variation in the time of occurrence and the degree of these regressive alterations. It has been our general observation that women who show rather prompt regression following the menopause usually have minimal subjective symptoms and that these are of short duration.

The hormonology according to the suggested theory of our group may be outlined as follows. When the ovaries, due to their decreasing function, cease to condition the pituitary and as a result the pituitary tends to show a compensatory hyperactivity, the adrenal cortex becomes active partly due to its sympathetic response to the hyperfunction of the anterior pituitary and partly due to the removal of direct antagonistic effects of the ovaries. This increased activity of the adrenal cortex is followed by an increased production of androgens. As the androgens increase, they tend to lower the hyperactivity of the pituitary. As a result, the pituitary becomes stabilized instead of continuing an unchecked and increasing course of hyperactivity. The stabilization is perhaps at somewhat higher levels than those which characterize the period of ovarian activity. This secondary stabilization of pituitary function may be the factor responsible for physiologic readjustment which constitutes the spontaneous cure of the subjective symptomatology of this period. According to this theory, as the androgens check pituitary hyperactivity, these androgens in turn become decreased in amount. This explains relatively low androgenic titers of women well past the menopause. This point of view suggests that as the ovaries fail in function, the adrenal cortex tends to take their place as important organs in regulation of pituitary function. Thus it may be said that the adrenal cortex becomes the gonads of the aged.

The effects of injections of testosterone propionate upon the androgenic titers of women show little significant difference between effects reported in the male. The percentage recovery of the injected material varied markedly from 71% to 7.6%. Other workers have reported similar variations. It may be significant that the highest percentage recovery was obtained from the smaller doses given over relatively long periods of time. Since the colorimetric method permits no inferences to be drawn in regard to qualitative alterations in the androgens, no data are available as to the chemical state in which testosterone appeared in the urine. The significance of recovering large amounts of androgens after therapy is not quite clear. Failure of the substance to appear in large quantities in the urine may indicate either utilization and exhaustion or frank destruction. The rapid appearance of large quantities in the urine may indicate that absorption was too rapid and that an excess of

the injected material was excreted as a waste product. Studies of androgenic titers in the blood if they were possible would permit more significant deductions.

Some evidence has been submitted that the injections of estrogenic substance tend to lower androgenic titers in the female.

No critical data have been reported upon the effects of injections of testosterone propionate upon the titers of estrogens and of the pregnandiol complex in normal women. Information of this character is most desirable. It would seem unwise to employ androgens in therapy of women until some definite information is obtained upon their effects on the hormonology of the normal female. Studies of this character are now in progress.

The diverse data which have been reported in this communication are regarded as constituting a survey of the studies done so far. Subsequent communications will consider larger numbers of patients in each of the various groups.

SUMMARY

Urinary androgens were investigated by a modification of the colorimetric method of Oesting in a group of 27 women who had diverse levels of ovarian function. In all, 672 24-hour specimens of urine were titrated. Daily titers of the same woman varied markedly. The use of average values of many daily determinations was deemed necessary for significant data.

Essentially normal androgenic titers were obtained in the following groups of women: *a*), 5 healthy women, aged 21 to 43 years, who experienced cyclic menses; *b*), 2 women, who had had operations involving hysterectomy with ovarian conservation and 5 women, who had had operations involving hysterectomy and bilateral oöphorectomy; *c*), 2 young women, aged 14½ and 19 years, who had statural and sexual retardation and postponed menarches; *d*), 2 young women, aged 17 and 29 years, who had functional menometrorrhagia associated with various degrees of endometrial proliferation and differentiation.

Androgenic titers tended to be high in the following groups of women: *a*), 4 women, aged 16 to 30 years, who had had episodes of amenorrhea which ranged in duration from 4 months to 5 years; *b*), 5 women, aged 42 to 74 years, whose last episodes of uterine bleeding had occurred from 9 months to 26 years prior to the period of observation.

Injections of testosterone propionate, which varied as to single doses from 10 to 100 mg. and as to number from 1 to 10, resulted in definite elevations of androgenic titers. The recoveries of the androgen injected ranged from 7.6% to 71.0%. Intensive estrogenic therapy of 2 patients caused definite decreases in androgenic titers.

The following generalizations are suggested by the data reported. The chief source of androgens in the female is not the ovaries, but most likely the adrenals. The complexional 'coloring' and the androgenic titers of women apparently are related. The increased androgenic titers of some patients who have episodes of amenorrhea may be of diagnostic, prognostic

and therapeutic import. An apparent increased production of androgens during the early post-menopausal period may signify the existence of another mechanism for stabilization of pituitary function after ovarian effects have become minimal. The androgens may play an active rôle in the production of the regressive alterations in the sexual system of ageing women.

We appreciate the kind and willing cooperation of the 27 women who made these studies possible. We acknowledge the efficient technical services rendered by Miss Louise McMillan and by Miss Elizabeth Wilkinson.

REFERENCES

1. Korenchevsky, V.: *Brit. Med. J.* 2: 896. 1937.
2. Callow, R. K.: *Proc. Roy. Soc. Med.* 31: 841. 1938.
3. Womack, E. B., and F. C. Koch: *Endocrinology* 16: 273. 1932.
4. Butenandt, A., and H. Dannenbaum; *Ztschr. f. physiol. Chem.* 229: 192. 1934; Butenandt, A., and K. Tscherning: *Ztschr. f. physiol. Chem.* 229: 167. 1934; Butenandt, A., H. Dannenbaum, G. Hanisch and H. Kudszus: *Ztschr. f. physiol. Chem.* 237: 57. 1935; Butenandt, A., K. Tscherning and H. Dannenbaum: *Ztschr. f. physiol. Chem.* 248: 205. 1937.
5. Callow, N. H., and R. K. Callow: *Biochem. J.* 32: 1759. 1938.
6. David, K.: *Acta brev. Neerland. de Physiol.* 5: 85. 1935.
7. Koch, F. C.: *The Harvey Lectures* 23: 205. 1937–1938.
8. Pederson-Bjergaard, K.: *Zentralbl. f. Gynäk.* 60: 372. 1936.
9. Kochakian, C. D.: *Endocrinology* 21: 60. 1937.
10. Dingemanse, E., H. Borchardt and E. Laqueur: *Biochem. J.* 31: 500. 1937.
11. Dorfman, R. I., W. W. Greulich and C. I. Solomon: *Endocrinology* 21: 741. 1937.
12. Chou, C. Y., and H. Wu: *Chinese J. Physiol.* 11: 429. 1937.
13. Oesting, R. B., and B. Webster: *Endocrinology* 22: 307. 1938.
14. Nathanson, I. T., L. E. Towne and J. C. Aub: *Endocrinology* 24: 335. 1939.
15. McCullagh, E. P., and J. F. Renshaw: *J.A.M.A.* 103: 1140. 1934.
16. Koch, F. C.: *J. Urol.* 35: 382. 1936.
17. Kenyon, A. T., T. F. Gallagher, D. H. Peterson, R. I. Dorfman and F. C. Koch: *J. Clin. Investigation* 16: 705. 1937.
18. McCullagh, E. P., J. M. Rumsey and W. K. Cuyler: *Proc. Cen. Soc. Clin. Res.* 11: 18. 1938.
19. Dorfman, R. I., and J. B. Hamilton: *J. Clin. Investigation* 18: 67. 1939.
20. Hoskins, W. H., J. R. Coffman, F. C. Koch and A. T. Kenyon: *Endocrinology* 24: 702. 1939.
21. Hamilton, J. B., and R. I. Dorfman: *Endocrinology* 24: 711. 1939.
22. Hamilton, J. B., and G. Hubert: *Science* 88: 481. 1938.
23. Hamilton, J. B.: *Anat. Rec.* 67: 22. 1937 (Abstract); Greenhill, J. P.; The 1938 Year Book of Obstetrics and Gynecology: Year Book Publishers, Chicago, 1939; Loeser, A. A., quoted by Foss, G. L.: *Lancet* 1: 992. 1938.
24. Dantchakoff, V.: *Compt. rend. Soc. de biol.* 124: 516. 1937; Greene, R. R., and A. C. Ivy: *Science* 86: 200. 1937; Hamilton, J. B., and W. U. Gardner: *Proc. Soc. Exper. Biol. & Med.* 37: 570. 1937; Foss, G. L.: *Lancet* 1: 992. 1938.
25. Wu, H., and C. Y. Chou: *Chinese J. Physiol.* 11: 413. 1937.
26. Oesting, R. B.: *Proc. Soc. Exper. Biol. & Med.* 36: 524. 1937. Oesting, R. B.: *Proc. Soc. Exper. Biol. & Med.* 39: 76. 1938.
27. Callow, R. K., and C. W. Emmens: *Biochem. J.* 32: 1312. 1938.
28. Zimmermann, W.: *Ztschr. f. physiol. Chem.* 233: 257. 1935; Zimmerman, W.: *Ztschr. f. physiol. Chem.* 245: 47. 1936.
29. Cuyler, W. K.: Unpublished data.
30. Hamblen, E. C., and W. K. Cuyler: *J.A.M.A.* 113: 38. 1939.
31. Hamblen, E. C., C. Ashley and M. Baptist: *Endocrinology* 24: 1. 1939.
32. Smith, G. V. S., and O. W. Smith: *Am. J. Obst. & Gynec.* 36: 769. 1938.
33. Hamblen, E. C.: *Endocrinology* 24: 13. 1939.
34. Simpson, S. L., P. de Fremery and A. Macbeth: *Endocrinology* 20: 363. 1936.
35. Cahill, G. F., R. F. Loeb, R. Kurzrok, A. P. Stout and F. M. Smith: *Surg., Gynec. & Obst.* 62: 287. 1936.

THE ESTROGENS OF URINE FROM WOMEN: HYDROLYSIS, SEPARATION AND EXTRACTION[1]

O. WATKINS SMITH, GEORGE VAN S. SMITH
AND SARA SCHILLER

From the Fearing Research Laboratory, Free Hospital for Women

BROOKLINE, MASSACHUSETTS

COHEN AND MARRIAN (1) were the first to attempt a quantitative separation of the estrogens of human urine. They demonstrated that the 'strong' phenolic fraction taken from an ether extract by means of N/10 NaOH contained the tri-hydroxyestrogen, estriol, whereas the 'weak' phenolic fraction, insoluble in N/10 NaOH but readily soluble in normal NaOH from toluene, contained the ketonic estrogen, estrone. The application of this method of separation to the study of estrogen metabolism in rabbits (2) and in both pregnant and non-pregnant women (3, 4, 5) has supplied information of physiological importance which could not have been gained by the estimation of 'total' estrogens.

In testing the completeness of the Cohen and Marrian separation of estrone and estriol fractions, we employed semicarbazide treatment (6) in order to determine whether any non-ketonic estrogen was taken over into the estrone fraction or any ketonic estrogen into the estriol fraction. (Ketonic estrogens are rendered biologically inactive by semicarbazide treatment through the formation of semicarbazones.) The consequent finding of non-ketonic estrogenic activity in the estrone fractions of all urines from women thus far so analyzed[2] has been reported elsewhere (4, 5), together with our reasons for believing that this so-called X estrogen portion may contain estradiol. The recovery experiments to be shown indicate that it does not contain estriol. The amounts of this material excreted appear to be of physiological interest and significance. Furthermore, semicarbazide treatment of estrone fractions gives a more accurate picture of estrone excretion.

The object of this paper is to present the final method which has been evolved and been found to be the most satisfactory for the hydrolysis of estrogenic esters in urine from women and for the separation and extraction of estriol, estrone and X estrogen, together with a brief account of the experimental results which have led to its adoption. A number of coincidental pertinent observations will also be discussed.

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[2] We have not yet employed semicarbazide treatment on urines from girls, from women after the menopause or from males.

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The method of bioassay used throughout this investigation has been previously described (3). Employing this technic with our strain of rats, 1 γ of estrone is equivalent to 1.5 to 2.0 R.U., 1 γ of estriol to 2.0 to 2.5 R.U. and 1 γ of estradiol to 20 to 25 R.U. These figures cover a seasonal variation which is checked by frequent standardization with the crystalline estrogens.

Hydrolysis of Conjugated Estrogens in Urine from Women

Investigation of the optimum conditions for acid hydrolysis of human urine was made and applied to the determination of urinary estrogens almost simultaneously in this laboratory, January, 1935 (7) and by Cohen and Marrian, December, 1934 (1). The importance of hydrolysis has been generally accepted, since it has been learned that the urinary estrogens are almost entirely excreted in combined forms. Various methods of hydrolysis have been published but, inasmuch as the exact forms in which the estrogens occur in human urine have not as yet been chemically identified, with the exception of estriol glucuronidate (8), it has been impossible to demonstrate whether or not a given method effects complete hydrolysis. The values resulting from the use of the Cohen and Marrian (1) and the Smith and Smith methods (7) are the highest, however, reported in the literature, and in a previous publication (3) these two procedures for hydrolyzing urine from women were shown to give practically identical values and to cause no change in the potency of added estrone or estriol.

Powdered zinc added to urine prior to acid treatment was found to yield marked augmentation of estrogenic potency (9). The indications are that this result is due, at least in part, to conversion of estrone to a reduced form of greater estrogenic activity, and to the hydrogenation of non-estrogenic substances, closely related to the estrogens, into forms which exhibit estrogenic activity. We have no complete proof that increased hydrolysis of conjugated estrogens may not also be involved.

A large percentage of the increase after zinc hydrolysis is in the estrone fraction. Furthermore, zinc hydrolysis of pure estrone (in a solution of high salt concentration) results in a 3 to 5-fold increase of the original estrogenic potency of estrone. Conversion of estrone, however, does not account for all of the increase in potency. The greatest proportional increase has been found in urines at the time of menstruation (9), when no estrone is present (4). Zinc hydrolysis (T_{zn}) of specimens from which all the estrogens freed after the regular acid hydrolysis (T_0) have been removed yields more estrogenic potency. One might interpret this as indicating that enhanced hydrolysis enters into the increase of T_{zn} over T_0. At the time of menstruation, however, when T_{zn} over T_0 is at its highest, the additional estrogenic potency which may be recovered by performing T_{zn} on T_0 residuals (in terms of percentage of total increase of T_{zn} over T_0) is at its lowest. Since neither estrone conversion nor increased hydrolysis accounts for the high T_{zn} values at the time of menstruation, we believe that estrogen breakdown products or estrogen precursors may be excreted in greater amounts at this time and may be rendered estrogenic by zinc hydrolysis. Zinc hydrolysis does not affect the activity of estriol or estradiol.

It is apparent, therefore, that the addition of zinc, even though yielding

higher values, is not applicable to the determination of urinary estrogens as such. Moreover, it has been observed that certain urines upon standing show a quite rapid increase in the estrogenic potency that may be recovered after acid hydrolysis. A number of specimens after standing for several months at room temperature have yielded values, on simple acid hydrolysis, 4 to 7 times as high as those given by hydrolysis when fresh, and, interestingly enough, values which checked exactly with those acquired by zinc hydrolysis when fresh. We suspect, therefore, that certain bacteria or enzymes may exert the same influence as zinc hydrogenation upon estrone and non-estrogenic steroids. Any hydrolysis that involves delaying extraction for any length of time is open to the criticism that estrogenic activity not accountable to the estrogens present at the time of excretion may have developed.

Until the urinary estrogen esters shall have been identified, the only criteria for a satisfactory method of hydrolysis must depend upon comparative results and the demonstration that the procedure which gives the highest values neither destroys added estrogens, converts them into more potent forms, nor makes estrogenic substances from closely related inactive materials. We have therefore continued to boil urine under a reflux condenser for 10 minutes after the addition of 15% concentrated HCl, since this procedure has been proven to fulfil all but the last of the above criteria and presumably fulfils this also. The reflux condenser is used to avoid evaporation, as higher concentrations of acid destroy estrone added to urine. The time is limited to 10 minutes and the specimen immediately cooled under the tap, since a longer heating period with 15% HCl involves destruction. (A 50% loss of pure estrone added to urine ensues if the specimen is kept at 100° C. for 1 hour in 15% HCl. The addition of powdered zinc prevents this destruction but, as noted above, introduces overestimates due to hydrogenation.) Any method which involves a long heating period with high acid concentration is open to this criticism. Cohen and Marrian employ a 2-hour heating period, but at the weak acidity called for by their procedure no demonstrable destruction of added estrogens occurs. We prefer the Smith and Smith method because of its greater convenience.

Separation of Urinary Estrogens

The Cohen and Marrian procedure for separating the 'strong' and 'weak' phenolic fractions consists of extracting the hydrolyzed urine with ether, washing the ether extract with Na_2CO_3, extracting the ether with N/10 NaOH to remove estriol, backwashing the N/10 with ether, evaporating the residual ether with the ether backwashings to dryness, taking the residue up in toluene and extracting the toluene with N NaOH to remove estrone. They make no claim that this separation is quantitative for estrone and estriol. Recovery experiments (table 1) in this laboratory, however, in which crystalline estrone, estradiol and estriol in varying amounts were added to aliquot portions of urines (whose 'estrone,' X estrogen and 'estriol' content were simultaneously determined) signify that their method for separating estrone from estriol is very satisfactory for all practical purposes. Semicarbazide treatment of the estriol fractions of urines from pregnant and non-

pregnant individuals have never been found to affect their estrogenic potency, indicating that no ketonic estrogen (estrone) is present in these fractions. Furthermore, no added estrone (demonstrable by bioassay) is soluble in N/10 NaOH from ether and taken into the estriol fractions (table 1, Exp. 3 A, 3 D and 6). In recovery experiments with estriol, no more non-ketonic estrogen (potency after semicarbazide) was found in the estrone fractions than was present without the addition of estriol, indicating that estriol is quantitatively taken out of ether by N/10 NaOH (table 1, Exp. 3 B, 3 D and 6). Recovery experiments with estradiol have shown that all but a very small percentage of this non-ketonic substance is insoluble in N/10 NaOH from ether and is taken over into the estrone fraction[3] (table 1, Exp. 2 B, 3 C, 3 D and 6).

Because of the satisfactory results from recovery experiments, so far as separation is concerned, by the Cohen and Marrian technic, we have made no attempt to modify their procedure for separation beyond adding the step of performing semicarbazide treatment upon all estrone fractions, thus being able to measure separately estrone and X estrogen. Strict adherence to accuracy would demand that these 3 fractions be termed the 'strong' phenolic, the 'weak' phenolic ketonic and the 'weak' phenolic non-ketonic estrogens, since it is entirely possible that other unidentified estrogenic substances may be present in all. For the present, however, we feel justified in referring to them as estriol, estrone and X estrogen.

Modified Procedure for the Extraction of Urinary Estrogens after Separation

Although the preceding experiments demonstrated that a satisfactory separation of estrone and estriol was accomplished by the Cohen and Marrian technic, the actual recovery of estrogens from each fraction was entirely inconsistent (table 1). Thus, the recovery of added estrone varied between 60 and 105%, of estriol between 33 and 103%, and of estradiol between 40 and 99%. There was no definite relationship between the percentage of recovery and the actual amounts or proportions of added estrogens. Nor did the volume of urine extracted appear consistently to play any part. In grouping these recovery experiments, however, according to whether the urine specimens used were collected during the first or second half of the menstrual cycle, it appeared that the unsatisfactory results were with those collected during the first half (table 1). We have found no explanation for this apparent effect of the time of the cycle upon extraction of estrogen. However, some modification which would eliminate, as much as possible, the dependence of recovery upon the type of urine seemed advisable.

In recovering the separated estrogens from the NaOH solutions, Cohen and Marrian recommend bubbling in CO_2 until the solutions are no longer alkaline to phenolphthalein prior to ether extraction. In following this step, two major difficulties have been encountered. (*a*) The amount of CO_2 gas necessary to render the N and N/10 NaOH solutions saturated to a point at which they were no longer alkaline to phenolphthalein varied with different

[3] This finding adds weight to the circumstantial evidence that X estrogen may be estradiol.

specimens. Carbon dioxide gas was bubbled for as long as 4 hours into some before this was accomplished; whereas, with others, 20 to 30 minutes of CO_2 treatment sufficed. The exact pH of the solutions after bubbling in CO_2 gas was not determined, but it must have varied considerably with different types of urine. It seemed very likely that the degree of acidity might influence the solubility of estrogens in ether. We therefore tried the effect of acidifying the NaOH solutions to pH 4 with HCl before ether extraction. The result (table 1, Exp. 4) was a somewhat better, but still unsatisfactory recovery of estrone and estradiol; whereas the loss of estriol was more marked when HCl was used, in place of CO_2 gas, prior to ether extraction. We next tried the effect of extracting the HCl acidified NaOH fractions with benzene for 24 hours in the Smith and Smith apparatus (7). This modification was used on the estriol fraction (table 1, Exp. 5), with a resultant 100% recovery, in spite of the fact that this specimen was collected during the first half of the cycle and showed low recovery in the estrone fraction when the unmodified Cohen and Marrian method was followed. In 6 different specimens from a normally pregnant woman the N NaOH extract from toluene (estrone fraction) was divided; one-third made acid to phenolphthalein with CO_2 gas and extracted with ether, one-third acidified to pH 4.0 with HCl and extracted with ether, and one-third acidified to pH 4.0 with HCl and extracted with benzene. The use of HCl and benzene, rather than CO_2 and ether or HCl and ether, resulted in a 35 to 100% increase in the estrogenic potency of the estrone fractions. In 2 other pregnancy urines the same experiment was tried on the N/10 NaOH extract from ether (estriol fraction) with the same outcome, that is, higher values with benzene extraction. This modification, therefore, appeared to give uniformly higher values.

(*b*) The use of continuous benzene extraction, after acidifying the NaOH solutions to a pH of 4.0 with HCl, not only made it possible to avoid the difficulty and uncertainty of CO_2 acidification but also eliminated the other major difficulty, namely, emulsions in extracting the acidified NaOH fractions with ether. These emulsions did not occur with all specimens, but when present were particularly troublesome in the estriol fractions.

Any emulsion formed in the first ether extraction of 'total' estrogens from hydrolyzed urines is usually taken down with the Na_2CO_3 washing. This is not always the case, however, particularly with specimens containing albumin. It was thought that all emulsions might be avoided if continuous benzene extraction were substituted for ether throughout the procedure. The separation of the estrogens from a benzene extract, however, is not quantitative. Even when the benzene extract is evaporated to dryness and taken up in ether prior to separation, the results are unsatisfactory, probably because benzene extracts more impurities which interfere with subsequent steps in the procedure. The first extraction of 'total' estrogens, therefore, is still made with ether.[4] When the emulsion is not eliminated by the Na_2CO_3

[4] That ether extraction of estrogens from urine itself to which 15% HCl has been added gives satisfactory recovery is demonstrated in table 1, Exp. 6. This is surprising, considering the loss of estrogens found when extracting from acidified NaOH solutions with ether. The explanation may lie in the high salt concentration of urine.

TABLE 1. RECOVERY OF ESTRONE, ESTRADIOL AND ESTRIOL FROM HUMAN URINE. ESTROGENS ADDED TO ALIQUOT PORTIONS OF URINES, THE ESTRONE, X ESTROGEN AND ESTRIOL CONTENTS OF WHICH WERE ALLOWED FOR IN CALCULATING PERCENTAGE RECOVERY. SMITH AND SMITH HYDROLYSIS, COHEN AND MARRIAN SEPARATION, AND COHEN AND MARRIAN UNMODIFIED VS. MODIFIED PROCEDURES FOR RE-EXTRACTING SEPARATED ESTROGENS FROM NaOH

Experiment	Estrogens perior to extraction						Urine specimen		Unmodified Cohen and Marrian NaOH acidified with CO_2 and extract with ether								
	Estrone R.U.		X Estrogen R.U.		Estriol R.U		Day of cycle	Volume used cc.	Estrone fraction R.U.			Estriol fraction R.U.			Recovery % of theortical		
	Present	Added	Present	Estradiol Added	Present	Added			Total	Ketonic Estrone	Non-Ketonic X	Total	Ketonic Estrone	Non-Ketonic Estriol	Estrone	Estradiol	Estriol
1	4	15	—	0	4	25	19 to 22	50	20	—	—	30	—	—	105		103
2A	6	200	14	0	10	200		515	170	160	10	133	—	—	78		63
2B	6	0	14	400	10	0	5 to 9	515	400	—	—	27	—	—		99	
															4% in estriol fraction		
3A	6	200	10	0	10	0		500	200	190	10	8	0	8	92		
3B	6	0	10	0	10	200	15 to 19	500	14	0	14	170	0	170			81
3C	6	0	10	200	10	0		500	200	0	200	14	0	14		97	
															2% in estriol fraction		
3D	6	200	10	200	10	200		500	400	200	200	170	0	170	97	95	81
4	0	250	0	250	0	5000	1 to 3	100	250	150	100	4000	—	—	60	40	80
5	<10	1000	<10	1200	<10	2000	1 to 3	570	1600	800	800	—	—	—	80	67	—
6	20	500	80	600	40	480	5 to 8	1000	925	375	550	170	0	170	72	81	33
4	Modification No. 1. NaOH acidified with HCl and extracted with ether								340	190	150	2800	—	—	76	60	56
5	Modification adopted. NaOH acidified with HCl and extracted with benzene								—	—	—	2000	—	—	—	—	100
6									1120	495	625	420	0	420	95	92	81

washing, centrifugation and repeated extraction of the residual is necessary. Once the first ether extract is clear, no further difficulties of this nature are encountered when the modification described above is employed, namely, continuous benzene extraction of HCl acidified NaOH fractions.

In order to determine whether or not the eradication of these two major difficulties also eliminated the loss of estrogens in certain urines when the unmodified procedure was used, estrone, estriol and estradiol were added to a large volume of urine collected during the follicular phase of the cycle and the results compared using both the unmodified and modified procedures (table 1, Exp. 6). This specimen happened to be one in which both the difficulties referred to above were particularly troublesome. Increased recovery of all 3 estrogens resulted from the use of the modified procedure. The recovery of estrone and estradiol was entirely satisfactory. There was a 19% loss of estriol, but with this particular specimen the loss of estriol when CO_2 and ether were employed was the greatest yet encountered, 67%. It seems probable, therefore, that the modified procedure may be counted upon, in any type of urine, to give a satisfactory recovery of estrone and estradiol and no more than a 20% loss of estriol. This loss is within the limits of error involved in the method of bioassay.

Non-Estrogenic Substances in Crude Extracts of Urine from Women which Inhibit the Action of Estrogens

In the curve for 'total' versus separated estrogens throughout a menstrual cycle (3), the values acquired during the second half for 'total' estrogens (hydrolyzed and extracted with benzene) were lower than the sum of the activities of the estrone plus estriol fractions (unmodified Cohen and Marrian procedure). Since employing the modified procedure for extracting the separated estrogens, we have found that higher values are acquired in the majority of pregnancy as well as non-pregnancy urines than are given by crude benzene extracts of hydrolyzed specimens. That this is not due to incomplete extraction with benzene has been demonstrated by the recovery of added estrogens with continuous benzene extraction. Benzene extracts of hydrolyzed urine contain androgens, and testosterone has been found to inhibit directly the effect of estrone upon the vaginas of spayed mice (10). The presence of androgens, however, cannot be held entirely responsible for the effect observed. No increased excretion of androgens has been noted during the second half of normal menstrual cycles (11). In a few specimens of pregnancy urine in which the difference between 'total' estrogen (crude benzene extract) and 'total' estrogen (estrone plus estriol) varied considerably, androgens were also measured.[5] The values for androgens bore no relation to the varying amounts of estrogen inhibitors which appeared to be present in the crude benzene extracts.

In the process of separating the urinary estrogens it has been found that each step in the procedure increases the estrogenic potency. In one pregnancy specimen, for example, the potency of the first ether extract was 16,000 R.U.

[5] Courtesy of Dr. I. T. Nathanson of the Collis P. Huntington Memorial Hospital, Boston, Mass.

per 24-hour volume. After washing with Na_2CO_3, this increased to 27,000, and the final total activity (estrone plus estriol fractions) amounted to 38,000 R.U. Obviously, estrogen inhibitors were being removed in the process of purification. In an attempt to simplify the procedure for recovering estrone, a portion of the residual ether, after removing estriol with N/10 NaOH, was titered and the result compared with the value obtained when the rest of the ether was evaporated to dryness, taken up in toluene, and reextracted with N NaOH. The latter gave a higher value than that obtained by the simpler procedure. It is apparent, therefore, that crude extracts of urine (either benzene or ether) contain substances which inhibit estrogenic activity and that the concentration of these substances is decreased by the various steps of the Cohen and Marrian procedure. We believe this inhibitory effect to be accountable to the presence in crude extracts of substances which have an affinity for the estrogen molecules and re-conjugate with them to form inactive materials. Certain extracts stored in alcohol have been found to lose potency upon standing. This potency has been temporarily regained by acidification with acid alcohol prior to the addition of olive oil for assay. Cohen and Marrian, in working out their method, were attempting to get extracts suitable for colorimetric assay. It appears that purification is equally important for bioassay, especially since urines appear to vary considerably in their content of inhibitory substances.

Unpublished experiments in this laboratory have revealed that pregnandiol, sodium pregnandiol glucuronidate and 2.7 dihydroxynaphthalene enhance the estrogenic effect of estrone upon the vaginas of spayed rats, probably through a protective action of these chemically related compounds against estrogen destruction. None of these particular substances could be present in the final extracts of the separated estrogens. However, it might be argued that other substances with a similar enhancing effect (cf. 12, 13) might be removed from the acidified NaOH fractions with continuous benzene extraction and account for the higher values for estrogens acquired with this modification of the Cohen and Marrian method. The presence of any appreciable amounts of substances which enhance estrogenic activity, however, would be detected by the finding of a decrease in potency with increasing purification of the crude extracts. As stated above, the exact opposite has been our experience. We believe, therefore, that the more satisfactory recovery with the modified procedure is entirely accountable to a more complete extraction of the separated estrogens.

Comparison of Bioassay with Colorimetric Assay

Some chemical method of measuring estrogens is highly desirable, considering the inherent inaccuracies and time consumption of bioassay. Pincus et al., working on this problem in 1936 (14), came to the conclusion that the phenolsulphonic acid test, after the method of Cohen and Marrian (1), gave reliable determinations of estriol fractions after the sixth month of pregnancy, whereas overestimates of hormone content in estrone fractions were the rule. In 1938 (3) comparison of colorimetric with bioassay was performed throughout pregnancy with specimens from a single individual, using the

same technic. Overestimation with colorimetric assay was consistently found in the estriol fractions, whereas the estrone fractions, after the second month, were practically free of inactive chromogenic materials.[6] These contradictory findings suggest that urines vary tremendously, both quantitatively and qualitatively, in their content of non-estrogenic materials that react with the color reagent. Considerable time has been spent in this laboratory in an attempt to find some method of eliminating or correcting for the overestimations with phenosulphonic acid. In this study the unmodified Cohen and Marrian procedure for separating and extracting estrogens was followed. The results of 13 comparative assays upon urines from 11 different pregnant women (table 2) made it apparent that no correction was at all feasible, due

TABLE 2. COMPARISON OF BIOASSAY WITH COLORIMETRIC ASSAY OF ESTROGENS IN URINES FROM PREGNANT WOMEN

Specimen		Period of gestation	Estrone fraction		Estriol fraction	
			Bioassay γ/24°	Colorimetric γ/24°	Bioassay γ/24°	Colorimetric γ/24°
M.B.	6/18/37	8 months	5100	4270+	24,500	39,979O
M.B.	6/22/37		1100	672U	10,600	38,000O
M.B.	6/25/37		1150	4140O	7,350	36,400O
D.P.	8/19/37	6 months	2500	3020+	10,700	10,800+
G.	8/28/37	15 days before delivery	6600	5400+	64,000	46,880U
L.R.	8/29/37	9 days before delivery	2500	3600O	21,300	42,080O
L.	9/26/37	41 days before delivery	800	1800O	10,700	14,000+
McC.	9/24/37	22 days before delivery	1500	3040O	8,000	11,600+
McF.	8/10/37	6 days before delivery	1335	2520O	21,200	34,080O
McG.	8/ 9/37	8 months	2450	2368+	11,800	30,710O
Sh.	9/30/37	7 months	1350	2254O	10,800	13,600+
Wy.	8/10/37	5½ months	1300	900U	—	—
L.W.W.	11/ 5/37	3 months	60	840O	146	3,960O

+ Within the limits of error. U Underestimation. O Overestimation.

to the fact that inactive chromogenic materials differed so widely in different individuals and even in different specimens from the same individual (e.g., table 2, *M.B.*). Our many attempts to remove pigment with decolorizors such as Lloyd's reagent and Norite resulted in large losses of the hormones. Moreover, we gathered the impression that urinary pigments were not the only interfering substances. Until some more specific color reagent for the estrogens is found, we feel forced, therefore, to depend entirely upon bioassay in measuring the estrogenic content of urines from both pregnant and non-pregnant women.

METHOD

Collection of Specimens

No preservative is used but the urines are kept in the refrigerator during the period of collection and hydrolyzed and extracted with ether on the day when the

[6] Contrary to expectation, the estrone values by colorimetric assay, in this individual, were considerably less than those determined by bioassay. This latter finding was taken to signify the presence in the estrone fraction of some estrogen of greater estrogenic potency than estrone, undoubtedly our more recently discovered X estrogen, possibly estradiol.

collection is completed. Careful instructions are given for obtaining an accurate 24-hour volume, and creatinine determinations are performed upon each specimen in order to detect any gross errors. If, in a normal adult, the 24-hour creatinine is less than 0.9 gm. it is fairly certain that some urine has been lost. When repeated specimens are being collected from the same individual, any gross errors may be readily detected and the true 24-hour volume roughly calculated, since the 24-hour creatinine excretion of a given individual is fairly constant.

The amount of urine hydrolyzed and extracted in non-pregnant patients varies between a 24-hour and a 48-hour volume, depending upon the estrogenic potency which may be expected. In analyzing pregnancy urines, one-half to one-tenth of a 24-hour volume is used, depending upon the period of gestation.

Hydrolysis

The urine is rendered approximately 1.5N by the addition of 15% concentrated HCl. It is then placed under a reflux condenser, brought to boiling and boiled vigorously for exactly 10 minutes. The hydrolyzed specimen is immediately cooled under running tap water.

Extraction and Separation

After cooling, the hydrolyzed urine is extracted 4 times with ether, using for each extraction an amount of ether equal to about one-fifth of the total volume of material being extracted. This ether extract is washed once with 20 to 30 cc. of 20% Na_2CO_3, this washing being discarded. The 'strong' phenolic fraction containing the estriol is removed from the ether by extracting 4 times with N/10 NaOH (25 to 30 cc. per extraction). The N/10 NaOH is washed once with 30 cc. of ether in order to remove any estrone that may have been extracted, this washing being added to the ether. The ethereal fraction is washed 3 times with 10 to 15 cc. of water and evaporated to dryness. The residue is transferred to a separatory funnel with 100 cc. of toluene, and the 'weak' phenolic fraction containing the estrone removed from the toluene by extracting 4 times with 25 to 30 cc. of normal NaOH.

The N/10 NaOH and the normal NaOH fractions are then each made acid to congo red with HCl and extracted for 24 hours in the Smith and Smith benzene extractor (7).

Pregnancy urine. The two fractions are then evaporated to dryness and the residues quantitiatively transferred with small amounts of 95% ethyl alcohol (and benzene if all the residue is not soluble in alcohol) to a test tube graduated at 20 cc. The extract is made up to volume with alcohol, tightly stoppered with rubber, and kept in the refrigerator to avoid evaporation. Aliquot portions are removed for bioassay, being added for this purpose to a measured amount of olive oil and put on the steam bath to remove the solvent. This is carried out in a wide-mouthed bottle large enough to permit frequent rotating during the evaporation so that no hormone will be left on the sides of the bottle.

Non-pregnancy urine. The estrone fraction is quantitatively transferred, after evaporating to dryness, to a test tube graduated at 20 cc. with small amounts of 95% ethyl alcohol and made up to volume with alcohol. Exactly 10 cc. of this is measured into a 125 cc. Erlenmeyer flask for semicarbazide treatment to determine X estrogen. The remaining 10 cc. is poured into a wide-mouthed bottle containing olive oil and the test tube is rinsed out with alcohol into the same bottle. All of the estriol fraction, after evaporating to dryness, is quantitatively transferred with small amounts of 95% ethyl alcohol to another wide-mouthed bottle containing olive oil. The two bottles are then placed on the steam bath to evaporate off the alcohol.

Semicarbazide treatment of estrone fraction for determining X estrogen (non-ketonic,

as yet unidentified). An aliquot portion of the estrone fraction in 95% ethyl alcohol (containing no more estrogenic potency than would be equivalent to 200 γ of estrone) is measured into a 125 cc. Erlenmeyer flask and diluted to 10 cc. with alcohol. (If benzene was used in washing out the benzene extract from the extraction flask before making up to volume, the accurately measured aliquot portion is evaporated to dryness in the 125 cc. Erlenmeyer flask and 10 cc. of 95% ethyl alcohol added.) Sixty milligrams of semicarbazide HCl and an equal amount of crystalline Na acetate are weighed into a small test tube, dissolved in 1 cc. of water and added to the alcohol. Refluxing is carried out for 5 hours. It is evaporated to dryness. The residue is taken up in 3 to 5 cc. of alcohol and the alcohol decanted from the precipitate into a wide-mouthed bottle containing olive oil. The precipitate is broken up with a stirring rod and washed several times with small amounts of alcohol which are decanted into the bottle. The alcohol is evaporated off on the steam bath.

SUMMARY

A satisfactory method for hydrolyzing urines from women and for the separation and extraction of estrone, estriol and an as yet unidentified non-ketonic estrogenic substance, X estrogen,[7] as described. The experimental results which have led to the adoption of this procedure are briefly reported. The Cohen and Marrian method (1) for separating estrone and estriol fractions has been adhered to. Bioassay, under standardized conditions, has been used throughout.

The separation of urinary estrogens not only gives information of physiological value that could not be gained by determining the total estrogenic potency but also higher values for 'total' estrogen than are acquired by the bioassay of crude extracts, apparently because of the removal, in the process of separation and purification, of substances which inhibit the action of estrogens.

The use of phenolsulphonic acid for the colorimetric assay of estrogens from urines of pregnancy has been found unreliable, due to the presence, in many specimens, of interfering substances which react with this color reagent and which we have been unable to remove without loss of estrogens. The concentration of these interfering substances varies tremendously, both qualitatively and quantitatively, with different urines, making any correction for overestimation impossible.

REFERENCES

1. Cohen, S. L., and G. F. Marrian: *Biochem. J.* 28: 1603. 1934.
2. Pincus, G., and P. Zahl: *J. Gen. Physiol.* 20: 879. 1937.
3. Smith, G. V., O. W. Smith and G. Pincus: *Am. J. Physiol.* 121: 98. 1938.
4. Smith, G. V., and O. W. Smith: *Am. J. Obst. & Gynec.* 36: 769. 1938.
5. Smith, G. V., and O. W. Smith: To be published.
6. Westerfield, W. W., and E. A. Doisy: *Ann. Int. Med.* 11: 267. 1937.
7. Smith, G. V., and O. W. Smith: *Am. J. Physiol.* 112: 340. 1935.
8. Cohen, S. L., G. F. Marrian and A. D. Odell: *Biochem. J.* 30: 2250. 1936.
9. Smith, G. V., and O. W. Smith: *Proc. Soc. Exper. Biol. & Med.* 36: 460. 1937.
10. Robson, J. M.: *Proc. Soc. Exper. Biol. & Med.* 35: 49. 1936.
11. Gallagher, T. F., D. H. Peterson, R. I. Dorfman, H. T. Kenyon and F. C. Koch: *J. Clin. Investigation* 16: 695. 1937.
12. Pincus, G., and N. Werthessen: *Proc. Roy. Soc. s.B.* 126: 330. 1938.
13. Emmens, C. W.: *J. Physiol.* 94: Proc. Dec. 10, 1938.
14. Pincus, G., G. Wheeler, G. Young and P. Zahl: *J. Biol. Chem.* 116: 253. 1936.

[7] On Aug. 21, 1939, we received word from Dr. E. A. Doisy that he had identified this estrogen, in material submitted by us, as dihydrotheelin (estradiol).

A COMPARATIVE STUDY OF THE EFFECTS OF MALE AND FEMALE SEX HORMONES ON THE HUMAN VAGINAL SMEAR

NORMAN O. ROTHERMICH

From the Department of Pathology, The Ohio State University, and the Columbus State Hospital

COLUMBUS, OHIO

ALTHOUGH the vaginal smear has been employed for a number of years as an index of female sex hormone activity, such study in the human being really had its foundation in the comprehensive work of Papanicolaou in 1933 (1). By following the daily smears of normal women throughout the menstrual cycle, he observed consistent alterations in the histologic pattern which enabled him to define specific criteria of vaginal response to hormone stimulus. He later extended these studies to castrate and menopausal women, describing the vaginal smear types characteristic of lack of hormonal influence, and then, with Shorr (2), used these observations as a guide to dosage for estrin therapy in the menopausal syndrome. Amplifying this on castrate women, Broun and I, collaborating with Werner and others (3), found that although the response to small doses of theelin was more delicate in the endometrium than in the vaginal smear, there was a definite parallelism between the two. In view of this and by virtue of its simplicity, the vaginal smear lends itself admirably to studies of the changes in the female genital tract.

With the advent of male sex hormone therapy in women, it becomes increasingly important to determine what effect, if any, this substance has on the female endocrine system. Long ago Moore and his group (4) provided the experimental background for such clinical therapeusis. From their extensive observations, the following conclusions were postulated: first, gonad hormones stimulate homologous reproductive accessories, but are without effect upon heterologous accessories; second, gonad hormones have no direct effect on the gonads of either the same or opposite sex and third, gonad hormones, of either sex, exert a depressing effect upon the hypophysis resulting in a diminished amount of the sex-stimulating factor available to the organism. If these principles were applicable to man, it would simply mean that the male sex hormone in women would have the same action as estrin on the pituitary, but would have no effect on the ovaries, uterus, vagina and breast. From a theoretical consideration then, this substance would be the ideal agent for treatment of such menopausal syndromes where it is desired to decrease the gonadotropic activity of the pituitary (5), without an unnecessary, and perhaps deleterious, effect on the secondary sex organs. Obviously before this form of therapy can be accepted, the premises outlined above must be conclusively demonstrated from the clinical viewpoint.

Since Moore's group predicated these conceptions much confirmatory (6, 7, 8)

and contradictory work (9, 10, 11) has been done on various animals. However, only recently have reports appeared concerning the use of testosterone in women. Geist, Salmon and Gaines (12, 13), confirming the earlier observations of Loeser (14) and Foss (15), reported an inhibitory influence of testosterone on the endometrium of human cyclical females and in cases of menorrhagia. As regards the effect on the vagina, Salmon (16) obtained 'positive,' that is follicular, vaginal smears after the injection of 400 mg. of testosterone propionate in a 46-year-old castrate woman whose previous smears had been of the atrophic variety. On the other hand, Papanicolaou also reporting on a single case (17) found exactly the opposite effect, namely, a change from the follicular type of smear to one characteristic of menopause. Furthermore, Shorr, Papanicolaou and Stimmel (18) demonstrated in menopausal women that the follicular smear obtained with injection of estradiol could be abolished by simultaneous injection of testosterone.

EXPERIMENTAL PROCEDURE

The purpose of this work was to attempt to demonstrate on a reasonably large series of cases what effect, if any, male sex hormone has on the human

TABLE 1. GROUP TREATED WITH MALE SEX HORMONE

Pt.	Age	Duration (mo.)		Total dose mg.	Appearance of smear	
		Menopause	Psychosis		Preliminary	With treatment
Z.A.	55	48	18	1395	atrophic	atrophic
H.B.	50	27	26	1185	atrophic	atrophic
L.B.	42	3??	2	1300	sl. foll.	atrophic
E.C.	46	24	12	1870	mod. foll.	atrophic
C.H.	47	7	3	810	mod. foll.	atrophic
H.K.	51	18	6	1360	atrophic	atrophic
E.M.	52	6	1	1250	sl. foll.	atrophic
I.O.	42	??	15	1030	mod. foll.	atrophic
M.R.	51	12	11	1520	mod. foll.	atrophic
C.W.	47	70(C)	16	890	atrophic	atrophic
A.W.	49	15	12	1060	atrophic	atrophic

vagina as revealed by the vaginal smear. For a more accurate appraisal of such effects, it was thought best to study simultaneously the smears of a similar group of women treated with an approximately similar dosage of estrogenic hormone. Because institutionalized cases offer greater opportunity for prolonged, continuous observation, the patients were selected from the involutional melancholia group at Columbus State Hospital. Twenty-two such women were divided into 2 groups as equally as possible on basis of age, duration of menopause and of psychosis, mental status quo and prognosis. Four patients had been castrated from 1 to 4 years before. All of the rest had passed the menopause at least 6 months previously, with the exception of 2 who had had occasional periods of bleeding at irregular intervals. The ages ranged from 40 to 56, with an average of 46.5. Vaginal smears were taken several times preliminary to treatment, and then at least once a week thereafter. In most of the cases smears were obtained daily for varying periods of time. The slides were fixed in ether-alcohol solution and stained with hematoxylin, eosin and water-blue, as recommended by Papanicolaou (1).

TABLE 2. GROUP TREATED WITH FEMALE SEX HORMONE

Pt.	Age	Duration (mo.)		Total dose I.U.	Appearance of smear	
		Menopause	Psychosis		Preliminary	With treatment
M.B.	56	50	24	400,000	atrophic	full foll.
E.B.	50	4+	5	510,000	atrophic	full foll.
O.M.	45	14(C)	6	700,000	atrophic	full foll.
M.N.	50	34	36	500,000	atrophic	mod. foll.
H.P.	55	6	4	650,000	sl. foll.	full foll.
M.S.	41	48(C)	4	209,000	atrophic	mod. foll.
F.S.	53	71	3	380,000	atrophic	full foll.
V.T.	40	16(C)	9	490,000	atrophic	full foll.
C.V.	47	12	12	500,000	sl. foll.	full foll.
L.W.	53	18	9	510,000	atrophic	full foll.
C.W.	47	20	6	330,000	mod. foll.	full foll.

In the one group, male sex hormone (oreton[1]) was given in doses of 35 mg. 3 times weekly for 5 weeks, following which the dosage was reduced to 25 mg. 3 times a week; after 6 weeks the dose was then adjusted according to the clinical condition. The total dose of oreton ranged from 810 mg. to 1870 mg. with an average of 1250 mg. The other group was treated with female sex hormone (theelin) with doses of 10,000 I.U. 3 times weekly, subsequent changes paralleling the oreton variations. All patients had received thorough physical and laboratory examinations, and the urine was assayed for gonadotropic hormone. This latter, as well as the therapeutic results, will be reported at a later date.

RESULTS

That some residual ovarian function persists long after menopause has set in is suggested by the fact that nearly half of these cases presented a vaginal smear characteristic of 'slight follicular' stimulation. This is in accord with the observations of Papanicolaou, Fluhman and others (2, 19, 20). Regardless of its appearance at the beginning, the vaginal smear in the group treated with theelin underwent the same change in all cases, being converted to a cornified or complete follicular type. The characteristics of this smear are sufficiently well known to obviate detailed description here.

Depending on the previous state of the vaginal mucosa, the appearance of the smear under oreton therapy varied. If there was complete atrophy beforehand, then the smear showed no change whatever following administration of oreton. The cornified and superficial cells remained entirely absent. Occasional small 'deep' cells with large, round nuclei were seen, usually together with many degenerated leukocytes, bacteria, debris and nuclear fragments, or degenerated cells with poorly defined cellular outlines. This picture persisted throughout the entire period of treatment.

On the other hand, if the preliminary smears showed any 'follicular' characteristics, as mentioned above, the injection of oreton resulted in a rather consistent alteration, leading toward atrophy. The epithelial cells lost their differentiated qualities and seemed to stick together in streaks and whorls. The mucus became more watery and less tenacious and the smear

[1] I am deeply indebted to Dr. Max Gilbert of the Schering Corporation for the generous supply of oreton.

rapidly assumed a 'dirty' appearance. After a variable period of time (from 1 to 3 weeks) the characteristics of the complete atrophic smear appeared and remained throughout the experiment. This was best exemplified in the 2 patients who had not had complete cessation of menses, and whose preliminary smears had shown definite estrin influence. In one case after the second injection, vaginal bleeding of 4 days duration occurred; at the end of this time the smear had changed to the atrophic character and persisted in that state together with the absence of further bleeding. In the other case the vaginal smear started to show regressive changes in a short time and reached the atrophic stage within 2 weeks. The patient has had no vaginal bleeding since the month preceding the initial injection and the smears have remained atrophic. Another case worthy of special mention is that of M. R. 51 years old, whose last menses occurred 11 months before. Her preliminary smears presented a moderate 'follicular' appearance which gradually underwent a retrogressive change with treatment. After a total of 175 mg. of oreton was administered, 12 days following the initial injection, vaginal bleeding began and lasted 3 days. Throughout the period of observation the subsequent smears were consistently of the atrophic type and no further bleeding occurred.

DISCUSSION

It is quite evident from these observations that male sex hormone has no stimulating effect on the vaginal epithelium in the doses given here, which in the light of other work certainly should be adequate. (One patient received 1200 mg. within a period of 45 days). That there may be a direct, local inhibitory effect remains a possibility, though this seems highly improbable in view of the fact that in those patients with an atrophic smear at the outset there was no perceptible change whatever. However, Hartman's excellent work (21) points strongly toward a direct, inhibiting influence, for he was able to prevent the bleeding which usually follows castration or cessation of estrin injections in monkeys by administration of testosterone.

Foss (15) suggests the possibility that male hormone counteracts and is a direct antagonist of the follicular hormone. This concept might be further supported by the regression of the follicular vaginal smear to the atrophic type. In fact most of the studies in the human being dealing with testosterone therapy have demonstrated endometrial atrophy in women with a normal or increased amount of available estrin. To prove or disprove the concept of intersexual gonad hormone antagonism, observations should be made while both androgen and estrogen are administered simultaneously in women with no ovarian function. In animals, experiments along this line have yielded results both for and against this concept (22, 23, 24), while in the human being, as stated previously, Papanicolaou (18) reported that estradiol in doses previously sufficient to produce vaginal cornification was unable to sustain the follicular smear when simultaneous injections of testosterone were given. As a further test of hormone antagonism, two patients, whose smears had shown good follicular response, were selected from the theelin-treated group and, after six weeks rest, they were given daily injections of male and female sex hormone simultaneously and in varying quantities. With

10 mg. of testosterone and 2000 I.U. of estradiol, the vaginal smear was persistently atrophic in appearance, but when the estradiol dosage was raised to 5000 I.U. the smear assumed a follicular character. However, adjusting the ratio to 25 mg. and 5000 I.U. respectively resulted in a prompt return of the atrophic type of smear. This was maintained in spite of a subsequent elevation of estradiol to 10,000 I.U. These findings would seem to indicate some degree of intersexual hormone neutralization.

The most attractive hypothesis to explain the action of male hormone in the female is (as Moore originally postulated) that it decreases the pituitary output of gonadotropic hormone in the same manner as estrin, thus affecting the ovary and secondary sex organs indirectly. Such a concept is well substantiated by the reports of Salmon (16) and Papanicolaou (18). My studies on the urinary excretion of gonadotropic hormone by these patients, while incomplete, thus far appear to bear out the pituitary-inhibition theory.

SUMMARY

The comparative effects of male and female sex hormones on the vaginal smear were studied in 2 groups of 11 menopausal women. Those receiving estrin responded with the typical follicular smear. In the testosterone-treated cases the vaginal smear, if atrophic beforehand, showed no change whatever; if previously follicular in character, the smear regressed to the atrophic type in which state it persisted. The stimulative effect of estrin could be entirely nullified by the simultaneous administration of adequate amounts of testosterone.

The results of this work warrant the following conclusions: (*a*) Male sex hormone has no direct stimulating action on the human vagina. (*b*) Male sex hormone nullifies, counteracts, or neutralizes the usual action of female sex hormone on the human vagina.

REFERENCES

1. Papanicolaou, G.: *Am. J. Anat.* (*suppl.*) 52: 519. 1933.
2. Papanicolaou, G., and E. Shorr: *Am. J. Obst. & Gynec.* 31: 806. 1936.
3. Werner, A. A., G. Jones, J. Roberts, G. O. Broun, C. Neilson and N. O. Rothermich: *J. A. M. A.* 109: 1027, 1937.
4. Moore, C., and D. Price: *Am. J. Anat.* 50: 13. 1932.
5. Albright, F.: *Endocrinology* 20: 24. 1936.
6. Robson, J. M.: *Proc. Soc. Exper. Biol. & Med.* 35: 49. 1936.
7. Browman, L. G.: *Proc. Soc. Exper. Biol. & Med.* 36: 205. 1937.
8. Zuckerman, S.: *Lancet* 2: 676. 1937.
9. Nelson, W. O., and T. Gallagher: *Science* 84: 230. 1936.
10. Salmon, U.: *Endocrinology* 23: 779. 1937.
11. Nathanson, I., C. Franseen and R. Sweeney, Jr.: *Proc. Soc. Exper. Biol. & Med.* 39: 358. 1938.
12. Gaines, J., U. Salmon and S. Geist: *Proc. Soc. Exper. Biol. & Med.* 38: 779. 1938.
13. Geist, S., U. Salmon and J. Gaines: *Endocrinology* 23: 784. 1938.
14. Loeser, A.: *Lancet* 1: 373. 1938.
15. Foss, G.: *Lancet* 1: 992. 1938.
16. Salmon, U.: *Proc. Soc. Exper. Biol. & Med.* 37: 488. 1937.
17. Papanicolaou, G., H. Ripley and E. Shorr: *Proc. Soc. Exper. Biol. & Med.* 37: 689. 1938.
18. Shorr, E., G. Papanicolaou and B. Stimmel: *Proc. Soc. Exper. Biol. & Med.* 38: 759. 1938.
19. Fluhman, C.: *Am. J. Obst. & Gynec.* 32: 612. 1936.
20. Shute, E.: *Endocrinology* 24: 744. 1939.
21. Hartman, C. G.: *Proc. Soc. Exper. Biol. & Med.* 37: 87. 1937.
22. Ihrke, I., and F. D'Amour: *Am. J. Physiol.* 96: 289. 1931.
23. Robson, J.: *J. Physiol.* 92: 371. 1938.
24. Zuckerman, S.: *J. Physiol.* 93: 15. 1938.

FURTHER OBSERVATIONS ON THE VAGINAL pH OF MONKEYS TREATED WITH ESTROGENIC SUBSTANCES

D. DOW AND S. ZUCKERMAN

From the Department of Human Anatomy, University Museum
OXFORD, ENGLAND

STUDIES ON WOMEN (1, 2) have indicated that the pH of the vagina falls as a result of estrogenic stimulation, and this conclusion was also reached in an investigation (3) of three rhesus monkeys (*Macaca mulatta*) that had been injected with estrone and whose vaginal pH was estimated by means of a glass electrode introduced directly into the vagina. It was not, however, in general agreement with observations on the normal cycle of the macaque (4) nor was it borne out by a series of 35 independent readings on 14 bilaterally gonadectomized rhesus macaques which were in various stages of estrogenic stimulation (5). Accordingly the following further observations were made on 12 gonadectomized rhesus monkeys, which were being used in collateral studies.

METHODS AND MATERIALS

The glass electrode method was used to estimate the pH of saline washes of the vaginal contents obtained by aspirating the vaginal cavity with a rubber tube attached to a 5 cc. glass syringe filled with normal saline. As far as possible, readings were made, immediately after the specimens were collected, every day during the period of observation of each animal.

Observations were made on animals which were given the following substances in courses of daily injections lasting 14 days. The figures in parentheses after the names of the preparations refer to the number of experiments through which the observations were made.

Estrone (8)
Estradiol (1)
Diethylstilbestrol (2).

Daily readings of the vaginal pH were also made on animals which were given 14 successive daily injections of progesterone after 14-day courses of estrogenic stimulation.

Estrone followed by progesterone (6)
Diethylstilbestrol followed by progesterone (1).

Observations were also made on animals which were given a single injection of one of the following estrogenic substances.

Estrone (1)
Estrone benzoate (4)

Estradiol (4)
Estradiol benzoate (2)
Diethylstilbestrol (3)
Diethylstilbestrol dipropionate (9).

In such cases readings were made daily until uterine bleeding supervened, or if no bleeding occurred, for a period of 40 days after the injection. Observations were also made on 3 spayed animals which were not under treatment, on a monkey immediately after bilateral ovariectomy, and on one into whose ovaries crystals of estrone had been implanted.

RESULTS

In 3 of the 11 experiments in which estrogenic hormone was administered daily for 14 days a marked and fairly steady fall occurred in the vaginal pH during the period of injection. In one animal which was given diethylstilb-

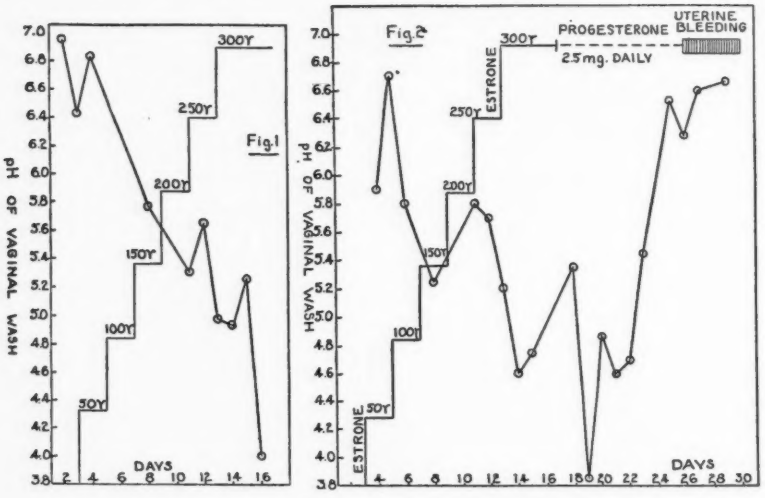

Fig. 1. The vaginal pH in a spayed rhesus monkey (203) injected for 14 days with increasing doses of diethylstilbestrol. Fig. 2. The vaginal pH in a spayed rhesus monkey (412) which was injected for 14 days with increasing doses of estrone and then for 9 days with 2.5 mg. of progesterone daily.

estrol the fall was from 7.0 to 4.0 (fig. 1). In 7 of the experiments the fall was both less extensive and less regular, the pH alternately rising and falling during the period of treatment, the fall being the more pronounced. In the eleventh experiment the results were anomalous, the pH falling from 6.7 at the start of injections to 4.8 on the ninth day, and then rising to 7.4 by the fourteenth day of injections. In this experiment, as in all except one of the 11 in this series, the amount of estrogenic hormone given daily was increased every 2 days during the course of treatment.

In 4 of the 7 experiments in which a course of injection with estrogenic hormone was followed by treatment with progesterone the vaginal pH under-

went a marked rise during the period of administration of progesterone, the maximum rise recorded being from 5.4 to 9.2. The rise in pH began within 2 to 6 days after the start of progesterone treatment (fig. 2). In 2 of the experiments an initial rise was followed by a fall. The results of the seventh experiment were altogether different in so far as the pH fell fairly consistently from 7.4 to 5.3 during the course of treatment with progesterone. This particular experiment was the one in which anomalous results were obtained during the period of estrogenic treatment.

Results varied in the experiments in which only a single injection of estrogenic hormone was administered. In cases in which sufficient hormone had been given to lead to uterine bleeding (an attempt was being made in these experiments to discover the smallest quantity of a variety of estro-

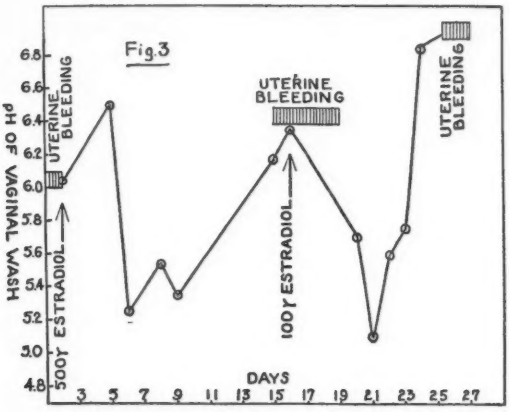

Fig. 3. THE VAGINAL pH IN A SPAYED RHESUS MONKEY (433) WHICH WAS GIVEN, IN 2 SUCCESSIVE EXPERIMENTS, SINGLE INJECTIONS OF ESTRADIOL (500γ and 100γ).

genic substances which, when given in a single injection, would lead to uterine bleeding) there was usually an initial fall in the vaginal pH (lasting in some cases for as many as 10 days), and a subsequent rise, which presaged the onset of uterine bleeding (fig. 3). As a general rule the period during which the pH rose was as long as the earlier period in which it fell.

As a rule, too, animals which were tested with different amounts of the same estrogenic substance showed a greater fall in pH as a result of larger doses of hormone. When too little hormone was given to induce bleeding no tendency towards a fall in pH was usually observed, and the pH fluctuated from day to day in the same way as it does in non-injected spayed animals. In such animals the daily fluctuation, while usually small, may sometimes be as much as 1.0, and it may be noted that the vaginal pH is frequently on the acid side.

The readings obtained from the animal into whose ovaries crystals of estrone had been implanted were in keeping with the general findings re-

corded above, the vaginal pH falling regularly during the period of observation (14 days) from 7.0 to 4.7. It was this animal which was subsequently gonadectomized, and after the operation the vaginal pH rose rapidly to 6.8.

A tendency for the pH to rise before uterine bleeding was observed in almost every experiment in which bleeding occurred.

DISCUSSION

The findings recorded above in general confirm the view that the pH of the vagina falls as a result of estrogenic stimulation, and in particular they corroborate the observations made on monkeys by Hall and Lewis (3). The individual records also show, as did those previously published (5), that the same degree of estrogenic stimulation is not necessarily associated with the same pH in different animals, or in the same animal at different times. Thus an amount of hormone (e.g. 5 mg. of diethylstilbestrol) which in one animal will induce a considerable change in the vaginal pH, may have little or no effect on another. It is the lack of such a relation which clearly lies behind the anomalous records which have been mentioned above. The divergence between our previously stated conclusion and the findings recorded in the present paper is also explained by this fact, for the records in the earlier study were collected with reference to different degrees of estrogenic stimulation and not with regard to the varying responses of individual animals. Why there should be no firm relation between the dose level of estrogenic hormone and vaginal pH remains to be determined.

SUMMARY

Day to day variations in the vaginal pH of 12 gonadectomized rhesus monkeys were determined by the glass electrode method during experiments in which the animals were being injected with either estrone, estradiol, diethylstilbestrol or estrogenic hormone followed by progesterone. Observations were made during 41 experiments. It was confirmed that the pH falls sharply when animals are under the influence of estrogenic stimulation and it was found that this effect is demonstrated as markedly by diethylstilbestrol as by natural estrogenic substances. It was also observed that the vaginal pH rises rapidly when injections of progesterone are given immediately after a course of daily injections of estrogenic hormone and that phases of uterine bleeding are generally preceded by a rise in the vaginal pH. The same degree of estrogenic stimulation is not necessarily associated with the same pH in different animals or in the same animals at different times.

We wish to express our thanks to Professor Sir Robert Robinson for his kindness in allowing us to use a glass electrode apparatus in his department. The monkeys were purchased with the aid of a grant to S.Z. from the Medical Research Council and the work was also supported by a grant to S.Z. from the Nuffield Medical Committee, Oxford, We also wish to express our thanks to Messrs. Ciba Ltd., British Drug Houses Ltd., and Schering Ltd. for the preparations used in this study.

REFERENCES

1. Schockaert, J. A. and G. Delrue: *Compt. Rend. Soc. de Biol.* 123: 306. 1936.
2. Lewis, R. M. and L. Weinstein: *Surg. Gynec. Obstet.* 63: 640. 1936.
3. Hall, B. V. and R. M. Lewis: *Endocrinology.* 20: 210. 1936.
4. Chen Mai, M. Y. and H. B. van Dyke: *Chinese J. Physiol.* 8: 203. 1934.
5. Ranson, R. M. and S. Zuckerman: *J. Physiol.* 89: 96. 1937.

BIOLOGICAL STUDIES OF THE GONADOTROPIC PRINCIPLES IN SHEEP PITUITARY SUBSTANCE[1]

HERBERT M. EVANS, MIRIAM E. SIMPSON, SIBYLLE TOLKSDORF[2] AND H. JENSEN[3]

From the Institute of Experimental Biology, University of California

BERKELEY, CALIFORNIA

IN A RECENT publication we described the preparation of two gonadotropic principles from sheep pituitary, namely a follicle stimulating hormone (FSH) and an interstitial cell stimulating hormone (ICSH) (1). A 100-fold increase in activity over the potency of the dried glands was achieved for each fraction and either was found to be free of the other component up to 10 to 20 times the M.E.D. We also briefly stated that our biological studies of these two gonadotropic fractions indicated that such physiological responses as synergism (activation), luteinization and antagonism can be demonstrated by injecting a combination of these two factors in certain proportions and by various routes. In the present communication we wish to report the results of these combination experiments.

Definition of Units and Standardization of FSH and ICSH

In order to be able to compare the biological results quantitatively we adopted the following physiological units for the two factors.

FSH. One rat unit represents the minimal total amount which, injected subcutaneously once daily over a period of 3 days into hypophysectomized rats (26 to 28 days old at operation, 6 to 8 days post-operative interval before injection), causes resumption of follicular growth,[4] as evidenced at autopsy 72 hours after beginning of injection.

ICSH. One rat unit represents the minimal total amount which, injected intraperitoneally once daily over a period of 3 days into hypophysectomized rats (26 to 28 days old at operation, 6 to 8 days post-operative interval before injection), causes repair of the deficient interstitial tissue of the ovary,[5] as evidenced at autopsy 72 hours after beginning of injection.

The hypophysectomized rats used in these studies were rigidly standardized in regard to age and post-operative interval. It was found that a post-operative period of

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[2] Present address: Squibb Institute for Medical Research, New Brunswick, New Jersey.

[3] Research Associate, March 1 to August 1, 1937, by invitation; December 1, 1937 to September 1, 1938, under a fellowship of E. R. Squibb & Sons, New York City. Present address: Squibb Institute for Medical Research, New Brunswick, New Jersey.

[4] By 'follicular growth' is meant the occurrence of healthy (non-atretic) follicles with small antra.

[5] By 'repair of the interstitial tissue' is meant the resumption of a normal nuclear picture and abolition of the wheel pattern of the nuclear chromatin which has given these cells the designation 'deficiency cells.' The repair of the nucleus is accompanied by increase of cytoplasm.

6 to 8 days is sufficient for the 'degeneration' of the interstitial cells portrayed by the nuclear change characteristic of 'deficient' interstitial tissue and for regression in the size of the follicles. The weight of the ovaries decreases during this interval from 20 mg. to 10 mg.

Our definition of units and methods of standardization are admittedly arbitrary. Histological study of the effect of these principles in hypophysectomized animals is necessary for accurate simultaneous determination of both the FSH and ICSH principles. The subcutaneous injection of FSH into hypophysectomized animals furnishes a more sensitive test for this principle than the subcutaneous injection into normal immature animals (tables 1, 2, 3). It is, however, true that the subcutaneous injection

TABLE 1. BIOLOGICAL STANDARDIZATION OF THE FSH FRACTION, NORMAL IMMATURE RATS, 72-HOUR TEST

Total dose		Subcutaneous injection			Intraperitoneal injection		
		Ovaries		Uterus	Ovaries		Uterus
		Weight	Autopsy diagnosis		Weight	Autopsy diagnosis	
mg.	R.U.	*mg.*			*mg.*		
0.025	1	21 20 20 20	mF mF sF	s m s	20 18 21 24	mF mF mF	s s s
0.05	2	31 30 28 24	sF mlF mF	s s s	18 22 20 21	s, mF sF mF	s s s
0.1	4	33 50 38 31	mF mlF mlF	m l s	34 28 30 27	CL lF, CL mF	m l s
1.0	40	129 104 102 73	lF, CL, do CL, do lF, do	m m l	26 40 35 39	lF lF, CL mF, CL	m l m

KEY TO ABBREVIATIONS

F, follicles or follicular
CL, corpora lutea
IT, interstitial tissue
R.U., rat units
do, distended oviduct
db, distended bursa
est, estrus
lut, luteinization or luteinized
def, deficient
dev, development
incr, increase
m, medium
s, small
l, large

of a combination of a purified FSH with prolan into normal immature animals gives a test of FSH potency comparable with that secured by the subcutaneous injection of this principle alone into hypophysectomized animals (table 3). But it may be pointed out that appreciable amounts of ICSH present in FSH preparations will render the prolan combination test less sensitive and that the prolan test gives no information regarding ICSH contamination. Since the ICSH principle exhibits no marked effects on the ovaries of normal immature rats when given at lower dose levels, it is necessary to test this principle in hypophysectomized animals (table 4).

It is well known that the quantitative outcome of the test depends not only on the number of injections, but also on the length of the test period. The interval between the last injection and autopsy is also important since the effects of both principles are transient. After a three-day interval, the ovaries of hypophysectomized rats show that in the case of FSH, follicular atresia has already taken place, whereas in the case of ICSH, regression of the interstitial tissue has again occurred.

EXPERIMENTAL

The biological experiments reported in this paper were carried out with FSH and ICSH fractions prepared according to the method recently published (1). The M.E.D. for the FSH preparation was 0.025 mg. as tested either subcutaneously or intraperitoneally in hypophysectomized rats, and that for the ICSH preparation 0.0125 mg. as tested intraperitoneally, or

TABLE 2. BIOLOGICAL STANDARDIZATION OF THE FSH FRACTION, HYOPHYSECTOMIZED IMMATURE RATS 72-HOUR TEST

Total dose		Subcutaneous injection			Intraperitoneal injection		
		Ovaries		Uterus	Ovaries		Uterus
		Weight	Histology		Weight	Histology	
mg.	R.U.	mg.			mg.		
0.025	1	12 12 11 12 7 11 16	sF incr; few mF; IT def	s s s s s s	15 17 14 14 8	sF incr; IT def	s s s s
0.05	2	16 16 16 21 21 25 22 31	s, mF incr; few mlF; IT def	s s s s s	17 21 11 15 10	s, mF incr; IT def	est s s s
0.10	4	33 44 49 24 35 22 38	mF many; few IF IT def	est est est m	15 13 10	sF, few mF; IT def	m m
0.25	10	71 52 32	s, m, lF many; few s, m, ml CL; IT repair partial	est est	39 46 52	s, mF many; s, m, ml CL many; IT repair	l est
0.5	20	67 62 56	s, mF many; s, m CL many; IT repair	est m	47 69 90	s, mF many; s, m CL many; IT repair	m est
1.0	40	39 43 51 54 70	s, m, mlF many; CL many; IT repair	est est est est	77 25 50 46 53	s, m, mlF many; CL many; IT repair	l l est est

0.05 mg. as tested subcutaneously in hypophysectomized rats (tables 1, 2, 3, 4).

FSH. In hypophysectomized animals the FSH exerted a specific effect, enlarging the ovarian follicles while leaving the interstitial tissue in the 'deficient' condition. Indeed, injections of 4 units over a period of 6 days into hypophysectomized animals did not disclose contamination with ICSH. When it was given at 10 times the M.E.D., or at higher levels, in the regular 3-day test, corpora lutea were formed with simultaneous partial repair of

the interstitial tissue. At levels of 20 to 40 R.U., approximately the same ovary weights were obtained on subcutaneous and intraperitoneal injections. In normal rats purified FSH preparations always elicit a higher response on subcutaneous injection. A possible explanation of this observation may be sought in the differences between the rates of absorption and excretion in normal as contrasted with hypophysectomized animals.

ICSH. As can be seen from the results in hypophysectomized rats (table 4), ICSH is more potent given intraperitoneally than subcutaneously; the

TABLE 3. COMPARISON OF DIFFERENT METHODS OF STANDARDIZATION OF FSH. SUBCUTANEOUS INJECTION, 72-HOUR TEST USING IMMATURE RATS

Total dose	Normal				Hypophysectomized		
	FSH			FSH +Prolan[1]	FSH		
	Ovaries		Uterus	Ovaries	Ovaries		Uterus
	Weight	Autopsy diagnosis		Weight	Weight	Histology	
mg.	*mg.*			*mg.*	*mg.*		
0.025	21	mF	m	73	12	sF incr;	s
	20 20	mF	s	38 61	12	mF;	s
	20	sF	s	73	11 12	IT def	s
					16		s
					7		s
					11		s
0.05	31	sF	s	95	25	s, m, lF many;	
	30 28	mlF	s	92 97	22	IT def	
	24	mF	s	104	16 20		s
					21		s
					19		s
					21		s
0.1	47	mlF	est	91	44	m, lF many;	est
	24 36	mF	l	135 107	49 35	IT def	est
	35	mlF	m	77	24		est
					22		m
Minimum Dose	0.05			0.025	0.025		

[1] Ovaries of animals receiving prolan only weighed 33 mg.

ratio was generally found to be 4:1. When injected alone intraperitoneally, even at high dose levels, no follicular development and no increase in ovarian weight was observed (see discussion of antagonism). On subcutaneous injection, at higher dose levels, 10 units or more, follicular stimulation and luteinization occurred. In the normal immature rat, low doses of ICSH, 1 to 5 R.U. subcutaneously, had no marked influence on the ovary (table 10). At higher dose levels with subcutaneous injection, the formation of corpora lutea and increase in ovary weight were observed.

Combination Experiments of FSH with ICSH and Prolan

Synergism. It is generally known that combinations of FSH with the pituitary LH factor given subcutaneously will show synergism as indicated

by augmentation of ovary weight brought about either by increased follicular development only or with simultaneous formation of corpora lutea (2). As already pointed out in the introductory paragraph of this paper, preliminary biological observations showed that the phenomena of both synergism and

TABLE 4. BIOLOGICAL STANDARDIZATION OF THE ICSH FRACTION, HYPOPHYSECTOMIZED IMMATURE RATS, 72-HOUR TEST

Total dose		Subcutaneous injection			Intraperitoneal injection			
		Ovaries		Uterus	R.U.	Ovaries		Uterus
		Weight	Histology			Weight	Histology	
mg. 0.0125	R.U.	*mg.*			1	*mg.* 10	IT repair partial	s
0.025					2	9	IT repair	s
0.05	1	7 7 9 13 9	IT repair partial	s s	4	19 11 16 19	IT repair	s
0.1	2	17 9 12 14 9	IT repair partial	s s s s	8	13 11 12 10 18 8	IT repair	s s s
0.2	4	11 9 13 13 18	IT repair partial	s s s s	16	15	IT repair	m
0.4	8	15 12 21 15 12	IT repair	s s s s	32	15 15 14	IT repair	m s
0.5	10	27	sF incr; few lF with lut walls; lT repair	m				
1.0	20	53	m, ml, lF many; lut walls; lT repair	m do				

antagonism were given by the combined administration of FSH and ICSH in the purity here recorded. We therefore undertook a quantitative biological study of the combined effects of these two principles and also a comparison of the similar response produced by the combination of FSH and prolan. The prolan used in our studies was a Squibb preparation, follutein.[6] The experimental conditions of the combination experiments with ICSH and with prolan, and the results, are indicated in tables 5 to 9, 15 to 17.

[6] It contained 200 R.U. per mg. standardized as follows: One R.U. was the minimal total dose which when given twice daily for 3 days subcutaneously, produced at autopsy 100 hours after onset of injection, corpora lutea in the ovary of normal immature rats 33 to 34 days of age at autopsy. The authors are greatly indebted to E. R. Squibb & Sons, New Brunswick, N. J., for the supply of follutein, prospermine, and gamone used in these studies.

TABLE 5. EFFECT OF COMBINATION OF FSH AND ISH, SUBCUTANEOUS INJECTION OF IN VITRO MIXTURE USING NORMAL IMMATURE RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Autopsy diagnosis	
		mg.		
		21	s, mF	s
	1	18 21	s, mF, mlF few	m
		24	mF	m
		18	s, mF	l
0	2	21 20	m. lF	l
		20	s, mF	m
		25	s, mF	l
	4	19 23	s, mF	est
		26	mF	m
		18	sF	s
	0	27 21	m, lF	m
		19	s, mF	s
		25	—	—
	1	16 18	s, mF	m
1		14	mF incr, mlF	m
		14	s, mF	m
	2	28 23	s, mF	m
		28	mF	m
		35	s, mlF	m
	4	31 34	lF, CL few	l
		35	mlF few	m
		33	lF	m
	0	29 28	s, mF	m
		23	s, mF	l
		28	mF incr	l
	1	31 28	mF	est
2		25	m, lF	est
		37	m, lF	est
	2	51 39	s, mF incr	est
		28	m, lF few	ml
		44	lF	l
	4	59 48	s, m, lF few	l
		40	s, mF	l
		59	mF	est
	0	30 43	mF	l
		40	sF incr	m
		27	lF	est
	1	32 46	s, m, lF	l
4		78	s, m, mlF	l
		56	lF	l
	2	86 76	lF, CL, do	l
		86	m, lF, CL few, do	l
		84	mF, CL	m
	4	52 59	lF, do	l
		42	s, mF incr	m

[1] Subcutaneous units.

Non-specific synergism. Some investigators have reported that an augmentative effect can be produced by the combined administration of certain gonadotropic pituitary preparations and various substances such as proteins and salts of certain heavy metals (2, 6, 7). We have studied the influence of zinc sulfate and casein on the physiological effect of our purified FSH and ICSH preparations (tables 10, 11).

TABLE 6. EFFECT OF COMBINATION OF FSH AND ICSH, SUBCUTANEOUS INJECTION OF IN VITRO MIXTURE USING HYPOPHYSECTOMIZED IMMATURE RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
		mg.		
1	0	12 12 12	sF incr, 1–2 mF; IT def	s s
	2	20 20 20	s, mF incr, lF few; IT normal	s s
	4	36 25 31	lF many, cysts; walls of some F lut; IT hypertrophy	est l
	8	38 31 35	mF many, mlF few; CL few; IT repair	est est
2	0	16 31 24	s, m, mlF incr; IT def	est s
	2	34 11 23	mlF; IT repair partial	est m
	4	70 50 60	lF many; CL; IT repair good	est, do est
	8	23 52 38	s, m, lF—walls lut; IT hypertrophy	m est
4	0	33 38 36	s, mF many; lF few; IT def	est est
	2	45 46 46	m, lF many; IT repair	est est
	4	56 49 53	lF many; cysts; m, lCL; IT repair	est est
	8	28 70 49	s, mF—walls lut; IT normal	est l
0	8	13 11 12	IT repair	s s

[1] Subcutaneous units.

Antagonism. It has been found that certain pituitary fractions administered intraperitoneally inhibit or decrease the effects of gonadotropic agents as portrayed by lower ovarian weights. Pituitary gonadotropic fractions themselves are usually less effective intraperitoneally than subcutaneously in regard to ovary weight. This phenomenon is called antagonism (3, 4). In order to elucidate the mechanism of this phenomenon we studied the

TABLE 7. EFFECT OF COMBINATION OF FSH AND ICSH, SIMULTANEOUS, SUBCUTANEOUS INJECTION OF BOTH FACTORS SEPARATELY USING HYPOPHYSECTOMIZED IMMATURE RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
2	4	mg. 40 33 37	m, lF—walls of few lut; IT repair	est est
	8	42 65 54	lF—walls lut; few CL; IT repair	est est
4	4	39 70 55	lF—walls of few lut; IT repair	est est
	8	37 51 77 70 59	m, lF; m, lCL; IT repair	l est l est
2	0	25 22 24	s, m, mlF; IT def	s s
4	0	33 38 36	lF; IT def	est est
0	4	18 15 17	IT repair; no F dev	s s
0	8	18 15 15 16 16	IT hypertrophy; no F dev	s s s s

[1] Subcutaneous units.

TABLE 8. EFFECT OF COMBINATION OF FSH AND ICSH, SIMULTANEOUS INJECTION OF FSH INTRAPERITONEALLY AND ICSH SUBCUTANEOUSLY USING HYPOPHYSECTOMIZED IMMATURE RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
2	4	mg. 25 11 18	sF incr; IT repair	m s
	8	22 25 24	sF incr; few mF; IT repair	est m
4	4	29 30 30	sF incr; IT repair	s s
	8	43 34 39	s, mF, one lF—walls lut; IT repair	est s
2	0	17 21 19	sF incr, few mF; IT def	est s
4	0	15 10 12	sF incr, few mF; IT def	m m
0	4	18 15 17	IT repair	s s
0	8	12 15 14	IT repair	s s

[1] Subcutaneous units.

effect of intraperitoneal injection of combinations of ICSH with either FSH or pregnant mare serum in normal and hypophysectomized animals (tables 12 to 14). The pregnant mare serum preparation used was an acetone precipitate, 25 mg. of which, given intraperitoneally to normal immature animals, produced an average ovarian weight of 125 mg. Fractions were generally tested for their antagonistic effect by in vitro mixture with this standard pregnant mare serum preparation and given intraperitoneally once daily for 3 days. The response was considered positive when at least a 25% reduction in ovary weight from the weight produced by pregnant

TABLE 9. EFFECT OF COMBINATION OF FSH AND ICSH, SUCCESSIVE, SUBCUTANEOUS INJECTION OF BOTH FACTORS SEPARATELY[1] USING HYPOPHYSECTOMIZED IMMATURE RATS

Total dose in R.U.[2]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
		mg.		
2	4	24 29 27	s, mF many, atretic; CL many; IT repair	m m
	8	29 24 27	s, mF few, atretic; CL few; IT repair	m m
4	4	106 134 120	s, mF few, atretic; lCL many; IT repair	l l
	8	72 72 72	s, mF few, atretic; lCL many; IT repair	m l
4	0	23 17 20	s, mF many, atretic; IT def	s s
0	4	21 12 16	IT repair	s s
	8	11 9 10	IT repair	s s

[1] FSH given day 1 to 3, followed by ICSH day 4 to 6, autopsy day 7.
[2] Subcutaneous units.

mare serum alone was observed. Since it was found that the response in regard to ovary weight varied greatly, it was necessary to use at least 6 rats in a group.

DISCUSSION

The studies of the effects of the follicle stimulating and the interstitial cell stimulating fractions of sheep pituitary in normal and hypophysectomized immature female rats permit certain general conclusions. Since we feel that one cannot a priori transfer biological observations from one sex to another and from one species to another, we prefer to restrict our interpretation and discussion of the various observations reported to the immature female rat.

Synergism. As can be seen from tables 5 to 9 augmentation of the ovarian response occurs in normal and hypophysectomized immature rats when FSH is injected with ICSH under the following conditions: *a*) when the two components are mixed in vitro and injected subcutaneously; *b*) when the

two components are injected subcutaneously at different sites; (c) when a preliminary injection of FSH is followed by ICSH, both given subcutaneously.

The synergism between prolan and FSH under different conditions is illustrated in tables 15 to 17.

TABLE 10. EFFECT OF COMBINATION OF ICSH AND $ZnSO_4$, SUBCUTANEOUS INJECTION OF IN VITRO MIXTURE, 72-HOUR TEST

	Total dose		Ovaries		Uterus
	ICSH	$ZnSO_4$	Weight	*Autopsy diagnosis*	
	R.U.[1]	*mg.*			
Normal rats	2	—	21	smF	m
			17	smF	m
			25 (18)	smF	l
			13	smF	s
			15	mF incr	m
			20	smF	m
	2	8	23	smF	est
			18	s, m, lF	est
			20 (21)	s, m, lF	est
			23	m, mlF	est
			23	many mF, 2–3 mlF	est
			21	smF, mlF	l
	4	—	21	mF incr, mlF	m
			27	sF	s
			18 (22)	sF	s
			28	sF, incr mF	m
			18	incr mF	l
			20	smF, few mlF	l
	4	16	30	smF, few lF	est
			21	mlF	est
			23 (31)	smF	est
			32	m, mlF, CL	est
			42	mF, CL	l
			36	m, mlF, 2–3 CL	l
				Histology	
Hypophysectomized rats	4	—	15 (16)	IT repair; no F dev	No dev
			17		
	4	16	10 (13)	IT repair; no F dev	No dev
			16		

[1] Subcutaneous units.

At certain dose levels of the ICSH factor (4 R.U.) formation of corpora lutea results on injection of the two factors under the conditions given above though either component given alone at these dose levels over a period of even 5 days will only show its single specific effect.

The augmentation of ovarian response consisting of increase in ovarian weight and formation of corpora lutea is apparently due to the two gonadotropic principles acting synergistically. Fevold, in collaboration with Hisaw and Greep, was probably among the first to submit evidence for the existence of two gonadotropic hormones in the anterior pituitary possessing synergistic properties, FSH and LH. Fevold reported that the LH fraction repaired the

TABLE 11. EFFECT OF COMBINATION OF FSH AND $ZnSO_4$ SUBCUTANEOUS INJECTION OF IN VITRO MIXTURE, 72-HOUR TEST

	FSH (4 R.U.)			FSH (4 R.U.)+$ZnSO_4$ (16 mg.)		
	Ovaries		Uterus	Ovaries		Uterus
	Weight	*Autopsy diagnosis*		Weight	*Autopsy diagnosis*	
	mg.			*mg.*		
Normal rats	41	mF	m	37	smF	m
	33 36	mF	m	37 35	m, mlF	m
	34	lF	m	32	mF	m
		Histology			*Histology*	
Hypophysectomized rats	24	m, lF	est	31	s, m, lF;	s
	22 23	IT def	m	18 24	IT def	s

TABLE 12. EFFECT OF COMBINATION OF FSH AND ICSH, INTRAPERITONEAL INJECTION OF IN VITRO MIXTURE USING HYPOPHYSECTOMIZED RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
		mg.		
1	0	15 16 17	sF incr; IT def	s s
	32	14 13 11	IT repair only	s s
2	0	11 10 15 17 21	sF inc, mF few; IT def	est s
	4	25 19 15	IT repair	m s
	8	15 15 15	IT repair	s s
	32	14 13 11	sF inc; IT repair	s s
4	0	15 13 10	sF inc, mF few; IT def	m m
	8	15 18 21	s, mF; IT repair	s s
10	0	39 46 52	s, mF many, lF few; s, m, l CL many; IT repair	l est
	32	45 35 25	s, mF many; s, m CL; IT repair	l m
20	0	47 69 90	s, mF many; CL many; IT repair	m est
	32	45 40 34	s, mF; CL many; IT repair	est l
0	32	15 15 14	IT repair only	s s

[1] Intraperitoneal unit for ICSH.

deficient interstitial tissue in hypophysectomized male rats, but apparently did not test it in hypophysectomized female rats (5). We have repeated his procedure for separating the two factors and found that his LH fraction which is considerably less potent per milligram than our purified ICSH will also repair the deficient interstitial tissue in the ovary of hypophysectomized

TABLE 13. EFFECT OF COMBINATION OF FSH AND ICSH, SIMULTANEOUS INJECTION OF FSH SUBCUTANEOUSLY AND ICSH INTRAPERITONEALLY USING HYPOPHYSECTOMIZED RATS, 72-HOUR TEST

Total dose in R.U.[1]		Ovaries		Uterus
FSH	ICSH	Weight	Histology	
		mg.		
2	4	15 10 13	IT repair	m s
	8	18 18	sF incr; IT repair	s
4	4	13 13 13	IT repair	s s
	8	33 16 25	IT repair	s s
2	0	16 16 16 31 21 21 25 22	s, m, lF; IT def	s m
4	0	44 49 22 38 35 24 22	s, m, lF; IT def	m est
0	4	19 11 16 19	IT repair	s
0	8	13 11 18 8 12 10	IT repair	s s

[1] Intraperitoneal unit for ICSH.

immature rats and will show synergistic and antagonistic effects when given in combination with FSH, depending on the experimental conditions.

The type of luteinization which results from the combination of a low dose of FSH with ICSH seems to be of thecal origin but we are not prepared to state that the granulosa can be completely excluded from participation in the formation of corpora lutea under these conditions. The luteinization secured by the injection of pregnant mare serum or a combination of prolan with low levels of a purified FSH is also thecal in character. On the other hand, granulosa initiation of the luteinization process is generally observed

when high doses of the purified FSH preparation are injected alone, but ultimate thecal participation is not denied. Whether the follicle stimulating principle as such can produce corpora lutea is still problematical; the luteinization observed may be due to contamination with ICSH. At present it is an open question whether corpora lutea of thecal and granulosal origin have the same functional properties.

Non-specific augmentation. Several investigators have reported that a number of substances which show no gonad stimulating activity when given alone, will enhance the response of hypophyseal gonadotropic preparations

TABLE 14. EFFECT OF COMBINATION OF ICSH AND PREGNANT MARE SERUM, INTRAPERITONEAL INJECTION OF IN VITRO MIXTURE USING NORMAL IMMATURE RATS, 72-HOUR TEST

Total dose		Ovaries		Uterus
ICSH	PMS	Weight	Autopsy diagnosis	
R.U.[1]	*mg.*	*mg.*		
		160	lF, CL	1
0	25	113 125	lF, CL	1
		91	m, lF	1
		108	lF, CL, do	est
2	25	85 92	lF, CL, do, db	1
		84	lF, CL, do	1
		77	mlF, CL, do	est
4	25	62 67	lF, CL, do	1
		62	mF, CL, do	1
		77	lF, CL	1
8	25	66 63	lF, CL	1
		46	lF, CL	1
		36	mF, CL	1
16	25	30 29	mF, CL	1
		22	mF, CL	1
		44	lF, CL	est
32	25	42 38	lF, CL	1
		28	mF, CL	m

[1] Intraperitoneal unit.

when injected in conjunction with them (2). This phenomenon has generally been attributed to a decrease in the rate of absorption of the active material. Some investigators (6, 7), however, interpret the augmentation effect of the pituitary LH fraction on FSH in the same way and, consequently, question the duality of the pituitary gonadotropic hormones. Saunders and Cole (7) have recently reported experiments which they consider as opposed to the conception that the LH factor has specific properties. The results reported in this paper give definite evidence that the ICSH (LH) factor possesses specific physiological properties, namely to stimulate the interstitial tissue and to act as a specific augmenting agent, when given in combination with the FSH factor under certain conditions. Our findings are, therefore, not in agreement with the non-specificity theory as expressed by these investigators.

TABLE 15. EFFECT OF COMBINATION OF FSH AND PROLAN, INJECTION OF IN VITRO MIXTURE USING NORMAL IMMATURE RATS, 72-HOUR TEST

Total dose in R.U.		Subcutaneous Injection			Intraperitoneal Injection		
		Ovaries		Uterus	Ovaries		Uterus
FSH	Prolan[1]	Weight	Description		Weight	Description	
		mg.			*mg.*		
		21	mF	m	20	mF	s
	0	20 20	mF	s	18 21	mF	s
		20	sF	s	24	mF	s
		79	lF, CL, do	l	54	mF, CL	l
	4	38 63	lF	est	40 41	lF	l
		73	lF, CL, do	l	39	lF, CL	l
		66	lF, CL, do	l	37	lF, CL	l
1	8	47 63	mF, CL, do	l	29 33	lF, CL	l
		75	sF, CL	l	34	lF	m
		63	lF, CL, do	l	36	sF, CL	l
	16	123 105	lF, CL, do	l	42 40	lF, CL, do	l
		129	lF, cyst, CL, do, db	l	43	lF, CL	l
		132	lF, CL	l	56	lF, CL, do	l
	32	106 144	lF, CL	m	68 60	lF, CL	l
		195	lF, CL, do	l	57	lF, CL	l
		31	sF	s	18	mF	s
	0	30 28	mlF	s	22 20	sF	s
		24	mF	s	21	mF	s
		111	lF, CL, do	l	22	sF, CL	m
	4	79 82	lF, CL, do	l	39 32	mlF, CL, do	l
		57	lF, cysts	l	36	lF	l
		104	lF, CL	l	45	mF	l
2	8	65 89	lF, CL, do	l	52 46	lF	l
		97	CL, do, db	l	40	lF, one CL	m
		231	Cysts, CL, do	l	51	lF, CL, do	l
	16	181 169	lF, cysts, CL	l	37 50	lF, do	l
		95	lF, CL, do	l	61	lF, CL	l
		146	lF, CL	l	62	lF, CL, do	l
	32	211 150	lF, CL, do	l	43 49	lF, CL	l
		92	lF, CL, do	l	43	mlF, CL, do	l
		33	mF	m	34	sF, CL	m
	0	50 38	mlF	l	28 30	mF, CL	l
		31	mlF	s	27	mF	s
		72	lF, CL, do	l	45	lF, CL	l
	4	123 95	lF, CL, do	l	27 38	mF, CL, do	m
		89	lF, CL, do	l	42	mF	m
		112	lF, CL, do	m	44	lF, CL	l
4	8	211 167	lF, CL	l	31 38	lF, CL	l
		177	lF, CL, do	l	40	mF, CL	l
		196	lF, CL, do	l	44	lF, CL, do	m
	16	146 160	lF, CL, do	l	57 46	CL	l
		138	lF, CL, do	l	43	mF, CL	l
		232	lF, cysts, CL, do	l	39	CL, do	l
	32	112 185	lF, CL, do	l	52 46	lF, CL	est
		210	lF, CL	l	46	lF, CL, do	l

[1] The unit of prolan as determined by the manufacturer was 0.005 mg. In the 72-hour test used here, in which 3 subcutaneous injections only were given, this dose was adequate to cause development of ovarian follicles and estrous uterus.

The possible synergic action of zinc sulfate and casein has been studied when given in combination with our two purified gonadotropic fractions. In the case of FSH, no augmentation occurred in normal or hypophysectomized immature rats (table 11). These observations seem to agree with the findings reported by Saunders and Cole that purified FSH preparations could

TABLE 16. EFFECT OF COMBINATION OF FSH AND PROLAN, SUBCUTANEOUS INJECTION OF IN VITRO MIXTURE USING HYPOPHYSECTOMIZED RATS, 72-HOUR TEST

Total dose in R.U.		Ovaries		Uterus
FSH	Prolan	Weight	Histology	
		mg.		
0	4	9 12 14	No F dev; IT repair	s s
	8	18 17 15	No F dev; IT repair	s s
0.5	0	12 13 13	No F dev; IT def	s s
	4	22 21 19	No F dev; IT repair good	s s
	8	26 21 16	No F dev; IT hypertrophy	l l
1.0	0	11 14 16	s, mF many IT def	s s
	4	29 33 36	s, m, lF many IT hypertrophy	est est
	8	52 49 45	s, m, lF many IT hypertrophy	est m
2.0	0	16 19 21	s, m, lF IT def	s s
	4	73 68 62	lF many IT hypertrophy	est l
	8	88 86 84	lF; m, lCL many CL cysts; IT hypertrophy	est est
4.0	0	33 36 38	s, mF many; lF few; IT def	est est
	4	64 64 63	m, lF; IT repair	est est
	8	86 66 46	m, lF; sCL few; IT hypertrophy	l est

not be augmented by casein, egg albumin and zinc sulfate. As can be seen from table 10, the addition of zinc sulfate to the purified ICSH fraction apparently causes an increased biological effect of the ICSH when given to normal immature rats as expressed in greater follicular development, occurrence of an estrous uterus and formation of corpora lutea. Similar though less pronounced effects were obtained in combination with casein.

In hypophysectomized animals, however, no augumentation effect was observed under the same conditions. Our findings regarding an apparent augmentative effect of either casein or zinc sulfate on purified ICSH preparations might explain the enhanced response shown by unfractionated pituitary gonadotropic preparations when administered in combination with these inert substances.

Antagonism. The synergistic property of the ICSH can only be demonstrated when it is injected subcutaneously in combination with FSH under certain conditions. On intraperitoneal injection of ICSH in combination with either FSH or with pregnant mare serum preparations, a decrease in

TABLE 17. EFFECT OF COMBINATION OF FSH AND PROLAN, SIMULTANEOUS SUBCUTANEOUS INJECTION OF BOTH FACTORS SEPARATELY USING HYPOPHYSECTOMIZED RATS, 72-HOUR TEST

Total Dose in R.U.		Ovaries		Uterus
FSH	Prolan	Weight	Histology	
		mg.		
2	4	33 50 66	lF; IT repair	est est
	8	46 44 42	m, lF many; IT hypertrophy	est est
4	4	44 61 77	m, lF; IT repair	est est
	8	69 72 75	lF; CL IT hypertrophy	est est
0	4	9 12 14	IT repair	s s
	8	18 17 15	IT repair	s s
2	0	25 24 22	s, m, mlF; IT def	s s
4	0	33 36 38	lF; IT def	est est

the FSH response results (tables 12 to 14). This antagonistic effect of ICSH can be observed in both normal and hypophysectomized animals if: *a*) ICSH and FSH or pregnant mare serum are combined in vitro and given intraperitoneally; *b*) ICSH is given intraperitoneally and FSH or pregnant mare serum subcutaneously. In this connection, it may be mentioned that a highly purified FSH preparation will also show an antagonistic effect against pregnant mare serum if injected intraperitoneally at proper doses. The mechanism of this antagonism is under investigation.

We also tested certain urinary gonadotropic preparations known to repair the interstitial cells in hypophysectomized animals by giving them to normal rats in combination with pregnant mare serum. In contrast to the hypophyseal ICSH fraction, they did not exhibit an antagonistic effect. The preparations studied are *a*) prolan (from pregnancy urine), *b*) gamone (from

menopause urine, containing both FSH and ICSH) and *c*) prospermine (from normal male urine, containing mainly ICSH and perhaps traces of FSH).

In spite of the fact that we have been able to show that the mechanism of the antagonist phenomenon consists of an inhibition or reduction of the follicle stimulating action of the FSH or pregnant mare serum given simultaneously, we are not in a position at present to claim any physiological significance for this phenomenon.

Since it is known that prolan, like ICSH, repairs the interstitial tissue in the hypophysectomized rat, we were interested in comparing these interstitial cell stimulating factors. At first glance, these two substances might appear to be identical. When injected alone for 3 days into hypophysectomized immature rats, both have as their sole effect the repair of the ovarian interstitial tissue, and both show synergistic action when given subcutaneously in combination with FSH in normal as well as hypophysectomized rats. Yet further comparison of these two principles shows definite biological differences. Prolan is a much more potent gonadotropic agent in normal animals than ICSH. The dose of prolan necessary to repair the interstitial tissue in hypophysectomized rats will cause follicular development and estrous uterus in normal rats whereas small doses of ICSH have no appreciable effect in normal immature rats. The interstitial cells, when stimulated by prolan, are larger and more eosinophilic than when stimulated by comparable units of ICSH. Finally, it may be pointed out again that prolan does not antagonize the follicle stimulating effect of pregnant mare serum when given simultaneously on intraperitoneal injection.

From the experimental data presented, it is evident that such physiological phenomena as *a*) synergism (activation), *b*) luteinization and *c*) antagonism can be demonstrated by the combined administration at certain adequate dose levels, of the pituitary FSH and ICSH factors under various modes of injection. One may perhaps raise the question whether the ICSH fraction here reported may still represent a mixture of the LH, ICSH and antagonist principles and that due to their similar physico-chemical properties no quantitative separation has been achieved in our chemical method of fractionation. Various other methods of purification and fractionation employed by us but which are not recorded here have always shown that the ICSH fraction produced, in combination with FSH, the phenomena of synergism, luteinization and antagonism in the same ratio, depending on the mode of administration. Until definite proof has been obtained that the various physiological effects described are produced by different principles, it seems premature to assume their separate existence (4, 8)[7].

[7] Since the present paper is essentially a condensed statement of facts, with recourse to a minimal amount of so-called interpretation, it may not be sufficiently clear to the reader why the assumption of the existence of a separate pituitary 'antagonist' was made in previous work. At that time the lower ammonium sulfate fraction, called here the hypophyseal ICSH, was assayed by the method of subcutaneous injection in hypophysectomized animals. It was known at that time that antagonism could be best demonstrated by intraperitoneal injection of the antagonistic fraction. As we have here shown, the ICSH is several times more effective given intraperitoneally than subcutaneously. In ICSH fractions standardized intraperitoneally, a striking parallelism exists in antagonistic properties and capacity to stimulate the interstitial cells. Similarly, the previous report that the interstitial cell stimulating fraction did not give luteinization when combined with FSH was due solely to inadequate knowledge of the importance of the route of injection and necessary quantitative relationships between these two hormones.

SUMMARY

The biological behavior of two purified gonadotropic pituitary fractions, FSH and ICSH, has been studied in normal and hypophysectomized immature female rats.

The phenomena of *a*) synergism (activation), *b*) luteinization, and *c*) antagonism can be produced by the simultaneous injection of these two factors under certain conditions.

The synergistic effect between the two factors is specific and is in contrast to the non-specific augmentation observed on the addition of certain substances (casein, zinc sulfate) to gonadotropic pituitary preparations.

The ICSH fraction when given intraperitoneally inhibits the follicular development produced by either FSH or pregnant mare serum.

Biological comparison between prolan and the hypophyseal ICSH principle indicates that they are different substances.

REFERENCES

1. Jensen, H., M. E. Simpson, S. Tolksdorf and H. M. Evans: *Endocrinology* 25: 57. 1939.
2. Van Dyke, H. B.: The Physiology and Pharmacology of the Pituitary Body, University of Chicago Press, Chicago, Ill., Vol. 2, 1939.
3. Leonard, S. L., F. L. Hisaw and H. L. Fevold: *Proc. Soc. Exper. Biol. & Med.* 33: 319. 1935.
4. Evans, H. M., K. Korpi, R. I. Pencharz and M. E. Simpson: *University Calif. Pub. Anat.* 1: 237. 1936.
5. Fevold, H. L.: *Cold Spring Harbor Symp. Quant. Biol.* 5: 93. 1937.
6. Maxwell, L. C.: *Am. J. Physiol.* 110: 458. 1934.
7. Saunders, F. J., and H. H. Cole: *Endocrinology* 23: 302. 1938.
8. Evans, H. M., K. Korpi, M. E. Simpson, R. I. Pencharz and D. H. Wonder: *Univ. Calif. Pub. Anat.* 1: 255. 1936.

EFFICIENCY OF EXTRACTION AND SEPARATION OF CERTAIN AP HORMONES[1,2]

A. J. BERGMAN, O. B. HOUCHIN[3] AND C. W. TURNER
From the Department of Dairy Husbandry, University of Missouri
COLUMBIA, MISSOURI

SINCE 1930 this laboratory has been engaged in a study of the physiological and chemical properties of the lactogenic hormone of the anterior pituitary (1–5). In 1935 the use of 60 to 70% alcohol at pH 9 to 10 as an extracting agent and the precipitation of the lactogenic hormone at pH 5.5 to 6.0 at an alcohol concentration greater than 86% was suggested (6). This extraction method was observed by Bergman and Turner (7) to recover the lactogenic hormone far more efficiently than earlier methods. As preliminary assays indicated that other AP hormones were also being extracted by this method, it seemed desirable to determine more precisely what hormones were included in this initial extract and how efficiently they were being recovered.

Recently a method for the separation of the lactogenic and carbohydrate metabolism hormones from the initial extract was described (8). Each of these fractions was shown to contain relatively little of the other hormone. In the present study, the efficiency of separation from other factors will be reported.

Methods of assay. The methods of assay for the various pituitary hormones which were used have been developed in this laboratory. The lactogenic hormone was assayed by the method of McShan and Turner (3) using the proliferation of the pigeon crop gland as a criteria of response. The carbohydrate metabolism hormone was assayed by the method of Bergman and Turner (9) which uses an increase of the blood sugar of male guinea pigs. The thyrotropic hormone was assayed with guinea pigs by the method of Bergman and Turner (10). The fat metabolism hormone was assayed by the method of Houchin and Turner (11) using the depression of the blood fats of the female guinea pig as an indication of response. The mammogenic hormone has been assayed by the method of Lewis, Turner and Gomez (12) using male mice.

For the gonadotropic hormone assay the day-old male White Leghorn chick is used. A unit of this factor is defined as the total amount of hormone administered over a 4-day period with subcutaneous injections once each day, which will elicit a mean weight increase of 50% (to about 13.2 mg.) in the gonads of 20 chicks weighing an average of 55 ± 10 gm. The normal gonad weight is about 8.8 mg.

EXPERIMENTAL

The cattle and sheep pituitaries used in this study were obtained at a

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[2] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 601.
[3] Contributed data on fat metabolism hormone.

packing house in Kansas City. They were frozen after collection and shipped to us on dry ice. The anterior lobes were separated by hand in a semi-frozen condition, thoroughly ground and an aliquot portion dehydrated with acetone, then washed with ether and dried. This dried powder was taken as as representative of the fresh pituitary.[4]

The two powders were then assayed for their content of lactogenic, thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones (table 1). It will be noted that the two types of pituitary are surprisingly similar in their content of all factors except the gonadotropic hormone, the cattle pituitaries containing only one-fourth as much of the latter.

TABLE 1. EFFICIENCY OF EXTRACTING THE HORMONES FROM SHEEP AND CATTLE PITUITARIES

	Sheep							Cattle						
Fraction	Acetone dried powder (A.D.P.)			Initial Extract				Acetone dried powder (A.D.P.)			Initial Extract			
Starting sample	1500 gm. fresh A.P. tissue			300 gm. A.D.P.				3300 gm. fresh A.P. tissue			660 gm. A.D.P.			
Amount recovered	300 gm. powder			39.861 gm. initial extract				660 gm. powder			105.765 gm. initial extract			
Recovery %	20			13.27				20			16.03			
Hormone Assay	One Unit	Units per gm.	Total Units Recovered	One Unit	Units per gm.	Total Units Recovered	Units Recovered	One Unit	Units per gm.	Total Units Recovered	One Unit	Units per gm.	Total Units Recovered	Units Recovered
	mg.			mg.			%		mg.		mg.			%
Lactogenic	1.9[1]	526.3	157,895	0.3	3333.3	132,870	84.2	2[1]	500.0	330,000	0.35	2857.1	302,186	91.6
Thyrotropic (guinea pig)	20.0[1]	50.0	15,000	2.0	500.0	19,930	132.9	20	50.0	33,000	2.50	400.0	42,306	128.2
Carbohydrate metabolism	37.5	26.7	8,000	4.0	250.0	9,965	124.6	40	25.0	16,500	4.00	250.0	26,441	160.2
Gonadotropic (chick)	4.0	250.0	75,000	0.4	2500.0	99,652	132.9	16	62.5	41,250	2.00	500.0	52,882	128.2
Fat metabolism	—	—	—	8.0	125.0	4,983	—	—	—	—	10.00	100.0	10,576	—

[1] Administered once daily in three injection sites rather than one.

The remaining portion of the fresh pituitary was then extracted by the method of Bergman and Turner (8) to the initial extract stage, in order to determine the efficiency of extraction of the various hormones. Assay of the hormones was repeated (table 1). On the basis of these assays it would appear that more than 100% of the hormones was being extracted by this method. As that is clearly impossible, an explanation was sought. If it occurred only in one or two cases, it might be considered as due to the variation in the biological assay of the hormones. As an alternative explanation, it was suggested that the hormones were not readily absorbed from the mass of pituitary tissue injected. As a result, the apparent amount of hormone present in the acetone-dried tissue was lower than that actually present. Upon extraction from the tissue, the hormone would be completely absorbed; and in comparison with the original tissue would appear to contain more than 100% of the hormone present in the starting pituitary tissue.

[4] Lewis and Turner (13) have recently observed that a 60% loss of mammogen occurred in acetone and ether drying of fresh pregnant cattle pituitary.

It was thought that if the daily amount of the acetone-dried tissue were injected at 3 points rather than one, better absorption might take place. It should be made clear that the 3 injections were made at the same time, and the results obtained are not due to spacing the injections at intervals during the day. In both lactogen and thyrotropic assays it was observed that the injection of the acetone-dried, pituitary powder in 3 places in the test animal caused a reduction in the amount of powder required to give a unit response (table 2). By this modification of the assay method instead of the initial extract indicating an extraction efficiency of more than 100%, it appeared that 84.2% of the lactogenic hormone was recovered from sheep pituitary and 91.1% from cattle pituitary. In the case of the thyrotropic hormone in sheep pituitary, even by the injection of the acetone-dried powder in 3 sites an efficiency of more than 100% in extraction is indicated. It will be noted

TABLE 2. EFFECT OF NUMBER OF INJECTION SITES ON HORMONE ASSAY OF ACETONE-DRIED PITUITARY POWDER

Hormone	Level of Injection	Pigeon Response	No. of Injection Sites
	mg.	%	
Lactogenic-sheep	2.5	50	1
	2.5	95	3
	1.9	50	3
Lactogenic-cattle	3.0	55	1
	3.0	100	3
	2.4	80	3
	2.0	45	3
	Level of Injection	Thyroid Weight	No. of Injection Sites
	mg.	*mg.*	
Thyrotropic-sheep	25	24.5	1
	25	28.6	3
	20	26.7	3

that rather large amounts are required for a unit. If this were to be injected at many sites so that absorption would be more efficient, the apparent content of hormone in the acetone-dried powder would be increased and efficiency of extraction reduced.

In spite of the difficulty in the assay of the acetone-dried pituitary, it seems reasonable to conclude that the initial extract contains a rather high proportion of the lactogenic, thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. Preliminary study indicates a marked depression of blood amino acid nitrogen following intraperitoneal injection of the initial extract in the dog. Therefore, the nitrogen metabolism factor will probably also be found present in considerable amount. Of the pituitary hormones which have been assayed in our laboratory, only the mammogenic hormone in pregnant cattle pituitaries does not appear to be recovered in the initial extract (13). Considering the fact that there is a reduction of 84 to 87% in the dry weight of the pituitary powder by this method of eliminating cellular debris and at the same time recovering a high percentage of well

TABLE 3. SEPARATION AND CONCENTRATION OF TWO FRACTIONS OF SHEEP AND CATTLE PITUITARIES

	Sheep								Cattle							
Fraction	Lactogenic Fraction				Thyrotropic and Others				Lactogenic Fraction				Thyrotropic and Others			
Starting sample	20.0 gm. Initial Extract				20.0 gm. Initial Extract				60.0 gm. Initial Extract				60 gm. Initial Extract			
Amount recovered	4.525 gm. Lactogenic				2.546 gm. Thyrotropic & Others				12.085 gm. Lactogenic				7.826 gm. Thyrotropic & Others			
Recovery %	22.63				12.73				20.14				13.04			
Hormone assay	One Unit	Units per gm.	Total Units Recovered	Units Recovered	One Unit	Units per gm.	Total Units Recovered	Units Recovered	One Unit	Units per gm.	Total Units Recovered	Units Recovered	One Unit	Units per gm.	Total Units Recovered	Units Recovered
	mg.			%	mg.			%	mg.			%	mg.			%
Lactogenic	0.15	6,666.7	30,167	45.3	8.0	125.0	318	0.5	0.15	6,666.7	80,567	47.0	10.0	100.0	783	0.5
Thyrotropic (guinea pig)	20.0[1]	Thyroids[2] Av. 19.6 mg.			0.25	4,000.0	10,184	101.8	20.0[1]	Thyroids[2] Av. 19.2 mg.			0.25	4,000.0	31,304	130.4
Carbohydrate metabolism	40.0[1]	Blood sugar[3] Av. 141.3 mg.%			1.60	637.5	1,591	31.8	40.0[1]	Blood sugar[3] Av. 135.7 mg.%			1.6	637.5	4,891	32.6
	100.0	10.0	45	0.9												
Gonadotropic (chick)	4.0[1]	Gonads[4] Av. 9.4 mg.			0.10	10,000.0	25,460	50.9	20.0[1]	Gonads[4] Av. 9.1 mg.			0.4	2,500.0	19,565	65.2
Fat metabolism	80.0[1]	Blood fat[5] Av. 43.7 mg.%			50.0	20	51	2.1	100.0[1]	Blood fat[5] Av. 43.8 mg.%			60.0	16.7	130	2.2

[1] The amt. injected represents 10 times the amt. of the Initial Extract required to produce a unit's resonse
[2] Normal guinea pig thyroids ave. 17.6 mg.—a unit increase (50%) equals 26.4 mg. calculate from equation
[3] Normal guinea pig blood sugar 110 mg. %—a unit increase (50%) equals 165 mg. %
[4] Normal White Leghorn chicks gonods average 8.8 mg.-a unit increase (50%) equals 13.2 mg.
[5] Normal guinea pig blood fat averages 60 mg. %—a unit decrease (50%) equals 30 mg. %

known hormones; the initial extract, therefore, should be very satisfactory for experiments requiring the presence of these hormones in approximately the proportions of the original pituitary. It may also serve as a starting point in an attempt to separate and further purify the hormones which the assay methods indicate are present.

A method has been described for the separation of the lactogenic hormone of rather high potency from the initial extract (8). Similarly a preparation containing the thyrotropic, carbohydrate metabolism and other hormones was obtained (table 3). These preparations have been assayed for the hormones previously described in order to determine their relative purity and efficiency of recovery.

The lactogenic hormone was found to be relatively free of other hormones present in the initial extract. When administered at a rate 10 times that required to equal a unit in the initial extract only slight evidence of activity was noted. In the case of the carbohydrate metabolism hormone in sheep extract, a 25-fold dosage (100 mg.) produced a unit response. While this lactogenic fraction is considered relatively pure, it is contaminated with slight amounts of other hormones and extreme caution should be exercised in assigning effects produced by these contaminants to the lactogenic hormone itself.

The most disappointing feature of this separation procedure is the apparent loss of lactogenic hormone in the process; less than 50% of the hormone in the initial extract appears to be recovered. Whether this is a real loss or is due to synergistic action of other hormones on the crop gland proliferation must await further study.

The thyrotropic hormone fraction was observed to be rich in carbohydrate metabolism and gonadotropic hormones as well. In fact the thyrotropic hormone appears to be recovered very efficiently by this method whereas only 50 to 60% of the gonadotropic and 30% of the carbohydrate metabolism hormones appears to be recovered.

Only minute amounts of the fat metabolism hormone were found present in either the lactogenic or thyrotropic fractions, thus confirming earlier observations by Houchin and Turner (14) in which these fractions were tested with rabbits. This appears to us as rather conclusive evidence that the fat metabolism hormone is not identical with any of the other hormones which we have assayed unless the effect is due to a synergism of certain hormones in the initial extract. The aqueous extract following the flavianic acid precipitation of the thyrotropic hormone fraction is now being investigated.

DISCUSSION

One of the major problems of pituitary hormone chemistry is to find means of efficiently extracting and separating the numerous hormones in the anterior lobe. Two general plans of attack have developed. Many investigators interested in one particular factor have worked out extraction procedures for extracting and concentrating this one factor without regard to other factors or to the presence of contaminating hormones. In this laboratory

it seemed desirable to attempt to find methods of extraction which would recover efficiently many if not all of the recognized AP factors as an initial extract, then attempt the separation and purification of the various factors which the assays indicated were present.

Data presented in this paper indicate that the initial extract accomplishes the first objective in that there is obtained a rather high proportion of the lactogenic, thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. At least some nitrogen metabolism hormone is also present. While entirely satisfactory adrenotropic assay methods have not been developed, there is qualitative evidence for its presence in our initial extract. If there is a separate entity which may be called the 'growth' hormone it is probably also present in this extract as Evans et al. (15) show that their most potent 'growth' hormone fraction is also rich in the lactogenic, thyrotropic and gonadotropic hormones. The writers prefer to believe that the hormone stimulus of growth is not vested in a single separate pituitary factor but rather results from a synergistic effect of several pituitary factors which influence directly and indirectly general metabolism and the metabolism of mineral matter, fats, carbohydrates and nitrogen. Of these probably the factor which comes closest to limiting growth is the nitrogen metabolism factor, especially in assays using normal female rats.

The mammogenic hormone which stimulates the growth of the mammary gland is not present in the initial extract. It is soluble in the lipid solvents and is removed by acetone, alcohol and ether. It can be recovered by the vacuum distillation of the acetone used in drying the fresh tissue or in the alcohol and ether solutions used in extracting and drying the initial extract.

The initial extract contains only 13 to 16% of the acetone-dried pituitary powder so a marked elimination of tissue debris is made by this method. For physiological experiments requiring the majority of the pituitary hormones in approximately their normal proportions and amounts, the initial extraction method should produce a satisfactory preparation.

The second phase of the problem, namely, the separation of the individual pituitary factors from the initial extract has been only partially successful. A lactogenic fraction has been shown to contain only traces of the other hormones while the thyrotropic fraction contains large amounts of the carbohydrate metabolism and gonadotropic and probably other hormones as well. Work is now in progress seeking chemical methods of separating this group of pituitary factors.

It appears that the fat metabolism hormone is not precipitated to any extent with flavianic acid. Its absence from the thyrotropic fraction indicates its separate identity and further indicates the possibility of its recovery in the aqueous fraction either by evaporation or other precipitating agent.

The cause of the apparent loss of considerable amounts of the lactogenic, gonadotropic and carbohydrate metabolism hormones is being sought. It is possible that a synergism exists between the hormones of the initial extract and upon separation their activity may be reduced.

SUMMARY

The efficiency of chemical extraction, separation and purification of certain hormones of sheep and cattle anterior pituitaries is reported. It was observed that by means of the initial extraction there was a reduction of 84 to 87% of the dry matter of the acetone-dried powder yet there remained a rather high percentage of the lactogenic, thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. Only the mammogenic hormone present in pregnant cattle pituitaries does not appear to be recovered in the initial extract.

The lactogenic hormone fraction separated from the initial extract by a method previously described was observed to contain only traces of the thyrotropic, gonadotropic, carbohydrate and fat metabolism hormones. However, slightly less than 50% of the lactogenic hormone appeared to be recovered by this method.

A fraction precipitated by flavianic acid was found to contain practically all of the thyrotropic hormone contained in the initial extract, whereas only 50 to 60% of the gonadotropic and 30% of the carbohydrate metabolism hormones appeared to be recovered. The fat metabolism hormone was not recovered to any extent. This is taken to indicate the separate identity of this factor.

REFERENCES

1. Turner, C. W., and W. U. Gardner: *Mo. Agr. Exp. Sta. Res. Bul.* 158, 1931.
2. Gardner, W. U., and C. W. Turner: *Mo. Agr. Exp. Sta. Res. Bul.* 196, 1933.
3. McShan, W. H., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 34: 50. 1936.
4. Gomez, E. T., and C. W. Turner: *Mo. Agr. Exp. Sta. Res. Bul.* 259, 1937.
5. Reece, R. P., and C. W. Turner: *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937.
6. Bates, R. W., and O. Riddle: *J. Pharm. & Exper. Therap.* 55: 365. 1935.
7. Bergman, A. J., and C. W. Turner: *J. Biol. Chem.* 118: 247. 1937.
8. Bergman, A. J., and C. W. Turner: *Endocrinology* 23: 228. 1938.
9. Bergman, A. J., and C. W. Turner: *J. Biol. Chem.* 123: 471. 1938.
10. Bergman, A. J., and C. W. Turner: *Endocrinology* 24: 656. 1939.
11. Houchin, O. B., and C. W. Turner: *Endocrinology* 25: 216. 1939.
12. Lewis, A. A., C. W. Turner and E. T. Gomez: *Endocrinology* 24: 157. 1939.
13. Lewis, A. A., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 39: 435. 1938.
14. Houchin, O. B., and C. W. Turner: *Endocrinology* 24: 638. 1939.
15. Evans, H. M., N. Uyei, Q. R. Bartz and M. E. Simpson. *Endocrinology* 22: 483. 1938.

PROGONADOTROPIC SERA OF ANIMALS TREATED WITH HYPOPHYSEAL EXTRACTS

P. A. KATZMAN, N. J. WADE AND E. A. DOISY

From the Laboratories of Biology and Biological Chemistry, St. Louis University

ST. LOUIS, MISSOURI

IN 1936 WE (1, 2) reported that the serum obtained from rats which had been treated with chronic implantation of pituitaries of the same species, enhanced the gonadotropic action of such hypophyses when both were administered subcutaneously in separate sites to immature female rats. The serum of normal rats did not produce this effect and the experimental serum alone did not induce gonadal stimulation. Augmentative activity was also induced in the serum of rats treated with an extract of rat pituitaries in place of the implants (3).

Subsequent to our first report, Collip (4) and Rowlands (5) have reported that the serum of sheep treated with sheep pituitary extract exhibits an enhancing effect on the gonadotropic action of this extract in immature female rats. The latter investigator found, as we had found with the rat serum, that the serum of the treated sheep possessed no gonadotropic activity when it was administered alone and that normal sheep serum did not augment the effect of the sheep pituitary extract. Collip (4, 6) found that the presence of this activity in the serum was temporary, being replaced by inhibitory activity after the sheep had been treated with the pituitary extract for about three months. Rowlands (5) treated a sheep for 140 days without observing inhibitory activity in the serum and Thompson (7) detected neither augmentation nor inhibition by the serum of sheep after 6 months of treatment.

The production of such progonadotropic sera is apparently not limited to the condition in which an animal is treated with pituitaries of the same species. Rowlands (5, 8) showed that the serum of a goat which had been treated with pig pituitary extract augmented the gonadotropic action of sheep, beef and pig pituitary extracts. The sera of rabbits injected with extracts of horse and beef hypophyses augmented pig pituitary extract. Thompson (9) found that sera of the dog and horse treated with sheep pituitary extract, temporarily at least, enhanced the gonadotropic effect of sheep pituitary extract. Spence, Scowen and Rowlands (10) reported that the sera of people who had been treated with an extract of pig pituitaries augmented the gonad stimulating power of this extract. In all instances mentioned the augmentation was produced in immature female rats. According to Rowlands (5) such an effect has not been produced in rabbits.

The present report consists of an extension of our work dealing with the chronic treatment of animals with homozoic pituitary extracts and a chemical study of the nature of the augmentative substance found in the serum.

EXPERIMENTAL

Animals Receiving the Chronic Injections

Sh 1 (ewe). Starting at about four months of age (body weight 62 lb.), this animal was injected subcutaneously daily with an amount of extract equivalent to 1230 mg. of acetone-desiccated whole sheep pituitary glands. This animal was killed on the 94th day of the experiment when it became apparent that it was dying of food poisoning or an intestinal infection.

Sh 2 (ewe); *Sh* 3 (ram). At the beginning of the experiment these animals weighed 62 and 72 lb. respectively and were 4 to 5 months of age. These animals which have thus far been treated for 262 days with sheep pituitary extract have received daily the equivalent of 1230 mg. of desiccated whole pituitary glands.

Sh 4 (ram), 5 to 6 months old, weighing 74 lb. has been treated with an extract of pig pituitaries as follows: 540 mg. of acetone-desiccated anterior lobes per day for 83 days, 1100 mg. daily for 59 days and 2200 mg. daily for 12 days (to date).

Pg 1 (boar), weighing 65 lb. was injected daily for 206 days with an amount of extract equivalent to 650 mg. of acetone-desiccated hog anterior lobes and for 158 days with the equivalent of 1100 mg. After this treatment (364 days) the animal (weight approximately 400 lb.) was killed and the experiment terminated.

Pg 2 (sow), 6 to 8 weeks old, weighing about 25 lb. has been injected daily with an extract of pig anterior lobes equivalent to 540 mg. of desiccated anterior lobes for 69 days; 1100 mg. for 64 days, and 2200 mg. for 10 days (143 days to date).

The extracts were prepared by repeatedly extracting the acetone-desiccated and powdered pituitary glands with 0.1 N $NaHCO_3$, neutralizing the extract with HCl, precipitating with 5 volumes of acetone, washing the precipitate with acetone and drying on a Buchner funnel. From the stock powder prepared in this manner, fresh extracts were made by extracting with 0.1 N $NaHCO_3$ and neutralizing with dilute HCl.

The sheep were bled from the jugular and the pigs from the ear veins.

Assay

The serum was tested in 21-day-old female rats weighing not less than 30 gm. Very few animals weighed over 40 gm. at this age and relatively few weighed as little as 30 gm. The pituitary extract against which the serum was tested was administered subcutaneously twice daily for 3 days in 6 equal doses. The serum was injected intraperitoneally at the same time and in the same way. Litter-mate controls received only the subcutaneous injections of the pituitary extract which was given in amounts sufficient to double or treble the weights of the ovaries of untreated animals. For each test 5 experimental animals and 5 litter-mate controls were used. All animals were autopsied 100 hours after the first injection. The ovaries and uteri were dissected, the latter freed of fluid by pressing between filter paper, and weighed. The magnitude of the decrease or increase of the average weights of these organs as compared with those of the litter-mate controls indicates the degree of inhibition or augmentation produced by the serum upon the activity of the pituitary extract.

In our earlier work the serum was injected subcutaneously in the side opposite to that in which the extract was injected. In the present work the serum has been injected intraperitoneally to avoid any possibility of the two mixing in subcutaneous spaces. The data in table 1 show, in agreement with Collip's (4) findings, that either route may be used. However, in our opinion the intraperitoneal injection is preferable. The slight augmentation obtained with the subcutaneous injection of the normal serum may have been due to mixing with the extract.

In preliminary experiments we have found that an augmented gonadotropic

TABLE 1. COMPARISON OF EFFECTS OF SUBCUTANEOUS AND INTRAPERITONEAL INJECTIONS OF SERUM TESTED AGAINST A STANDARD DOSE OF SUBCUTANEOUSLY INJECTED SHEEP PITUITARY IN IMMATURE FEMALE RATS

Sheep serum	Injection	Deviation from controls*	
		Ovaries	Uterus
		mg.	mg.
Normal	Subcutaneous on opposite side	+ 1.9	+11.1
	Intraperitoneal	− 2.3	+ 0.7
Sh1 S1	Subcutaneous on opposite side	+ 5.4	+11.0
	Intraperitoneal	+ 6.8	+15.6
Sh1 S2	Subcutaneous on opposite side	+ 1.2	+17.6
	Intraperitoneal	+16.7	+26.8
Sh1 S3	Subcutaneous on opposite side	+16.4	+31.3
	Intraperitoneal	+16.9	+39.1

* In this and the subsequent tables each value represents the difference between the average weights of the respective organs of 5 experimental animals injected with serum and extract and 5 litter-mate controls injected with an equal amount of the same extract alone.

response is also produced if the extract is injected intraperitoneally and the serum subcutaneously.

Effect of the Chronic Injections on the Serum

The treatment of the sheep with sheep pituitary extract has not resulted in the production of inhibitory substances in their serum in spite of the continued treatment for 262 days (table 2). In sheep *Sh 1* and *Sh 3* the aug-

TABLE 2. ENHANCING ACTION OF SERUM OF SHEEP TREATED WITH SHEEP PITUITARY EXTRACT ON THE GONADOTROPIC ACTION OF THE SAME EXTRACT IN IMMATURE FEMALE RATS

Days of treatment	Quantity serum injected	Sheep (*Sh1*)		Sheep (*Sh2*)		Sheep (*Sh3*)	
		Deviation from controls					
		Ovaries	Uterus	Ovaries	Uterus	Ovaries	Uterus
	cc.	mg.	mg.	mg.	mg.	mg.	mg.
0	3.0	− 2.3	+ 0.7	+ 4.2	− 8.5	+ 1.9	−10.5
33	2.4	+ 5.4	+11.0				
43	3.0			+ 3.3	+ 1.3	+19.5	+50.1
47	2.4	+17.7	+26.8				
54	2.1	+16.9	+39.1				
57	3.0			+ 4.4	+ 9.2	+30.4	+42.9
67	2.1	+28.9	+40.0				
71	3.0			− 6.5	− 2.1	+24.3	+56.1
82	2.1	+28.6	+40.5				
86	3.0			+ 1.5	+ 2.1	+15.1	+47.4
94	2.1	+20.0	+41.8				
99	3.0			− 4.4	− 6.1	+13.8	+44.4
124	3.0			+ 8.4	+26.4	+23.4	+59.9
139	3.0			+10.1	+22.4	+21.4	+44.3
160	3.0			+ 2.8	+39.5	+11.5	+51.9
183	3.0			+ 7.6	+63.5	+17.4	+78.9
195	6.0[1]; 1.5[2]			+14.2[1]	+51.0[1]	+ 9.6[2]	+69.5[2]
219	3.0[1]; 1.5[2]			+11.4[1]	+61.3[1]	+26.8[2]	+58.0[2]
239	1.5			+ 8.7	+38.4	+31.1	+41.7
262	1.5			+ 6.1	+41.0	+ 6.1	+58.7

[1] Indicates the volume of serum of *Sh2* used and the effects produced.
[2] Indicates the volume of serum of *Sh3* used and the effects produced.

TABLE 3. EFFECT OF SERUM OF SHEEP (*Sh4*) TREATED WITH PIG PITUITARY EXTRACT ON PIG AND SHEEP PITUITARY EXTRACTS IN IMMATURE FEMALE RATS

Days of treatment	Fraction of serum used	Whole serum or its equivalent	Pig pituitary extract		Sheep pituitary extract	
			Deviation from controls			
			Ovaries	Uterus	Ovaries	Uterus
		cc.	mg.	mg.	mg.	mg.
0	Whole	3.0	− 1.9	− 4.7	− 4.9	− 0.4
13	Whole	3.0	− 4.5	− 9.7	+ 3.9	− 0.9
28	Whole	3.0	−14.1	−12.2	− 2.1	− 1.9
49	Whole	3.0	− 2.1	− 5.9	− 6.0	−10.9
	Euglobulin	3.0	+14.1	+20.8	− 6.2	− 8.7
	Pseudoglobulin	3.0	+ 0.9	+ 6.7	−12.9	− 5.0
72	Whole	3.0	+ 3.7	− 8.6	− 1.1	−22.4
	Whole	3.0	+ 1.5	+ 7.2	+ 7.7	+ 2.9
108	Whole	4.5	− 4.2	−10.0	− 2.0	−20.5
	Whole	4.5	− 2.7	−30.9		
	Euglobulin	4.5	− 1.1	− 6.9		
	Pseudoglobulin	4.5	− 4.5	− 3.1		
128	Whole	6.0	+ 3.5	+ 6.1	− 2.9	0.0
149	Whole	6.0	−10.4	−14.8	−11.0	−4.4

mentative substance made its appearance before the 50th day of the treatment, while *Sh* 2, receiving the same treatment did not show this reaction until the 124th day. In no case has there been an apparent decrease in the activity of the serum in spite of continued treatment.

During the first 4 to 5 months of the treatment with the sheep pituitary extract, the testes of the ram (*Sh* 3) increased in size to approximately 6×3 in. (each testis). At the present time they have decreased to about 3×2 in. It should be remembered that this decrease in testicular size has occurred in spite of the continued presence of progonadotropic activity in the serum of this animal. We have been unable to detect any inhibitory activity in the pseudoglobulin fraction (9, 12) of the serum. Thus far this animal has continued to manifest sexual activity by constantly mounting another ram in the same pen.

The serum of *Sh* 4 receiving the pig pituitary extract has not as yet shown a consistent inhibitory or augmentative effect when tested with either sheep or pig pituitary extract (table 3). Moreover, neither type of activity has been consistently found in the euglobulin or pseudoglobulin fraction of the serum. In a few scattered instances appreciable augmentative and inhibitory effects have been obtained, but since these have not been reproducible with either the same sample or succeeding samples of serum it is doubtful whether any significance can be attached to them. The testes of this animal have slowly but steadily continued to increase in size during the experiment.

The chronic treatment of the pigs with pig pituitary extract has failed to induce progonadotropic activity in the sera of these animals as determined by testing the serum against pig pituitary extract in immature female rats (table 4). The serum of the boar (*Pg* 1) failed to show consistently appreci-

able effects, although an inhibitory response seemed to be more predominant toward the end of the experiment. The serum of the female (Pg 2) seems to possess some inhibitory properties toward the pituitary extract especially when measured by the effect on the uterine response. However, this has not been consistent and in addition the serum failed to alter appreciably the effect of the extract on the weight of the ovaries of the immature female rats. For these reasons the inhibition is not at all convincing.

TABLE 4. EFFECT OF SERUM OF PIGS INJECTED WITH PIG PITUITARY EXTRACT ON THE SAME EXTRACT IN IMMATURE FEMALE RATS

Days of treatment	Quantity of serum	Pig (Pg1)		Quantity of serum	Pig (Pg2)	
		Deviation from controls			Deviation from controls	
		Ovaries	Uterus		Ovaries	Uterus
	cc.	mg.	mg.	cc.	mg.	mg.
0	2.4	+2.2	+ 3.8	3.0	+8.4	− 9.7
27	2.1	+0.6	− 1.0			
29				3.0	+2.1	− 4.3
42	3.0	−5.1	− 2.6			
63	2.4	+1.4	− 9.8			
69				3.0	−7.5	−35.2
79				4.5	−7.9	−36.0
79				3.0	−9.3	−31.8
84	2.6	+5.0	+ 1.5			
92				3.0	−4.9	− 8.3
92				4.2	−1.5	−16.6
97	2.4	−1.3	+ 4.6			
106				3.6	−6.2	− 6.1
106				4.8	−3.7	−37.3
112	3.6	+0.8	− 3.6			
133	3.0	+6.4	− 1.3			
141				4.5	+4.7	+ 5.7
170	4.8	+2.8	− 9.3			
205	3.0	−0.5	+ 7.8			
219	3.3	+5.1	+ 5.6			
240	3.0	−6.3	− 6.9			
262	3.0	0.0	−13.5			
287	3.0	−3.1	−10.4			
321	3.0	−6.8	−33.4			
345	6.0	−0.5	− 2.6			
345	6.0	−2.1	+10.0			
364	6.0	−0.1	−37.3			
364	6.0	−8.0	−19.0			

The testes of the boar increased rapidly in size during the first half of the injection period and became abnormally large for an animal of that age. At the end of the experiment they appeared to be of normal size, weighing 725 gm. The seminal fluid contained an abundant amount of motile sperms, indicating that the testes were functional.

Effect of the Augmentative Substance of Sheep Serum Administered Alone and with Various Gonadotropic Extracts

In our earlier publications we (1, 2) showed that with the progonadotropic serum which was obtained from rats treated with implants of rat pituitaries no effect was produced on the gonads of the immature female

rats when the serum was administered alone. The same observation was made by Rowlands (5) and Collip (4) with the serum of sheep treated with sheep pituitary extract. We have repeated this work with the sera obtained from our sheep. As shown in table 5, the sera, which injected intraperitoneally enhanced the gonadotropic activity of subcutaneously injected sheep pituitary extract, injected alone had no appreciable effect on the weight of the ovaries and uteri. The control animals which received neither serum nor extract were litter mates of those which were injected only with serum.

Furthermore, the ovaries of adult female rats injected subcutaneously for as long as 11 days and those of virgin rabbits injected intravenously on 3 successive days with a potent augmentative preparation of sheep serum,

TABLE 5. INFLUENCE OF AUGMENTATIVE SUBSTANCE ALONE ON OVARIES AND UTERI OF IMMATURE FEMALE RATS

Sheep serum	Amount injected	Quantity sheep pituitary extract injected	Organ weights		Remarks
			Ovaries	Uterus	
	cc.	mg.	mg.	mg.	
Sh1 S5	2.1	13.5	68.8	89.5	Averages of groups of 5 litter mates
	0	13.5	40.2	49.0	
	2.1	0	13.3	19.8	Averages of groups of 5 litter mates
	0	0	14.3	16.3	
Sh3 S11	1.5	27.0	57.7	116.6	Averages of groups of 5 litter mates
	0	27.0	31.9	58.6	
	3.0	0	12.4	24.2	Averages of groups of 5 litter mates
	0	0	13.3	19.2	
Sh3 S13	1.5	27.0	70.7	104.4	Averages of groups of 5 litter mates
	0	27.0	39.6	62.7	
	6.0	0	12.9	22.8	Averages of groups of 5 litter mates
	0	0	14.3	22.1	

showed no apparent changes in weight or macroscopic appearance from those of uninjected controls. It therefore does not appear likely that the enhancing action is brought about by the stimulation of the hypophyses of the assay animals to produce an abnormal amount of gonadotropic hormone. Of course, to establish this point more firmly, hypophysectomized female rats should be used.

The data in table 6 substantiate the findings of Rowlands (5) that, at least within certain limits, the degree of augmentation depends upon the amount of the progonadotropic substance administered, when the quantity of pituitary extract is constant. In addition they show that a given amount of the active substance is relatively more effective in augmenting the effect of a small quantity of the pituitary extract. We wish to point out that the smallest amount of pituitary extract (4.5 mg.) did not produce an increase in the weight of the ovaries and uteri of the control animals. However, the same amount of extract injected in conjunction with a potent preparation

TABLE 6. EFFECT OF VARYING DOSE OF AUGMENTING SUBSTANCE AND OF PITUITARY EXTRACT

Sheep serum preparation		Sheep pituitary extract	% Increase above controls[1]	
Number	Whole serum or its equivalent		Ovaries	Uterus
	cc.	mg.		
	1.5	4.5	115.0	230.0
	1.5	6.75	190.0	105.0
Sh1 S6E2 Euglobulin	1.5	13.5	101.2	104.0
	1.5	45.0	40.8	76.8
	0.6	13.5	44.3	69.5
	1.5	13.5	101.2	104.0
	3.0	13.5	96.0	134.0
Sh1 S5E Euglobulin	0.3	13.5	33.8	3.2
	0.6	13.5	90.5	87.0
	3.0	13.5	114.8	129.6
Sh3 S8 Whole serum	0.9	27.0	18.3	38.3
	1.5	27.0	24.1	77.6
	3.0	27.0	93.7	114.3

[1] Control groups of 5 litter mates, injected with the extract alone, were used for each dose of the extract listed in column 3.

of the serum produced a considerable response. Such a low level of pituitary extract is the most sensitive for measuring augmentation. We have not used it routinely because when augmentation is not obtained one cannot rule

TABLE 7. INFLUENCE OF THE SHEEP SERUM ON VARIOUS GONADOTROPIC EXTRACTS

Sheep serum preparation		Gonadotropic extract		Deviation from controls	
Number	Amount injected	Source	Amount injected	Ovaries	Uterus
	cc.		mg.	mg.	mg.
Sh1 S5	2.1	Sheep pituitary	13.5	+28.6	+40.5
Sh1 S6	2.1	Sheep pituitary	13.5	+20.0	+31.9
Sh1 S6E2[1]	1.5	Sheep pituitary	13.5	+43.3	+54.4
Sh1 S4	2.1	Sheep pituitary	13.5	+28.7	+40.0
Sh3 S5	3.0	Sheep pituitary	13.5	+15.1	+47.4
Sh1 S5	1.5	Rat pituitary	4.8	+22.2	+24.9
Sh1 S6	2.1	Rat pituitary	4.8	+10.3	+17.1
Sh1 S6E2	1.5	Rat pituitary	4.8	+17.8	+23.1
Sh3 S5	3.0	Rat pituitary	4.8	+18.1[2]	+43.0[2]
Sh1 S5	2.1	Pig pituitary	22.5	+ 5.0	+ 8.4
Sh1 S5	1.5	Pig pituitary	18.5	− 4.5	+ 2.0
Sh3 S5	3.0	Pig pituitary	27.0	− 2.6	− 3.3
Sh1 S5	2.1	Beef pituitary	60.0	+ 1.6	+ 7.1
Sh1 S6E2	1.5	Beef pituitary	120.0	− 0.3	+ 2.0
Sh1 S5	1.5	Pregnancy urine	0.225	+ 9.4	+ 9.8
Sh1 S5	1.5	Pregnancy urine	0.225	+ 0.2	+ 9.9
Sh1 S4	2.1	Castrate urine	9.0	− 2.3	+16.4
Sh1 S5	2.1	Castrate urine	6.0	− 3.0	+18.4

[1] Solution of euglobulin fraction.
[2] 10 experimental animals and 10 litter-mate controls were used instead of the customary 5.

out inactivation of the pituitary extract without testing it against a preparation of known augmentative activity.

Rowlands (5) reported that the progonadotropic sheep serum did not enhance the gonadotropic action of extracts of pig, horse and beef pituitaries, of pregnancy urine or of pregnant mares' serum. We have confirmed these results with the extracts of pig and beef pituitaries and the urine of pregnant women (table 7). The serum, however, was found to augment considerably the gonadotropic action of rat pituitary extract. This effect is therefore not entirely species specific. With the gonadotropic material of the urine of

TABLE 8. EUGLOBULIN AND PSEUDOGLOBULIN FRACTIONS OF SHEEP SERUM TESTED WITH SHEEP PITUITARY EXTRACT

Sheep serum	Fraction	Whole serum or its equivalent	Deviation from controls	
			Ovaries	Uterus
		cc.	mg.	mg.
Sh1 S4	Whole	2.1	+28.9	+40.0
	Euglobulin	6.0	+47.2	+63.2
	Pseudoglobulin	6.0	− 1.1	+ 7.5
Sh1 S5	Whole	0.6	+21.2	+48.4
	Whole	0.3	+ 7.9	+13.5
	Euglobulin	1.2	+28.6	+39.7
	Euglobulin	0.6	+12.4	+ 3.2
Sh1 S6	Whole	2.1	+20.0	+41.8
	Euglobulin	3.0	+43.3	+54.4
	Euglobulin	1.2	+17.2	+36.0
	Pseudoglobulin	15.8	+ 5.7	+ 8.9
Sh3 S9	Whole	1.5	+ 8.1	+39.2
	Euglobulin	1.5	+16.6	+21.3
	Pseudoglobulin	12.0	+ 5.0	+19.7
Sh2 S11	Whole	3.0	+14.2	+51.0
	Euglobulin	3.0	+23.7	+40.3
	Pseudoglobulin	12.0	+ 4.7	+20.8
Sh3 S13	Whole	1.5	+31.1	+41.7
	Euglobulin	1.5	+25.4	+59.5
	Pseudoglobulin	10.8	+18.7	+30.2

castrated women no augmentation of the weight of the ovaries was observed but the uterine weights seem to be somewhat elevated. We have not determined the significance of a change of the weight of the uterus occurring without a parallel change of the ovaries but in the present instance we do not attach much importance to the discrepancy. The first section of table 7 shows that the preparations used with the various gonadotropic preparations were potent in augmenting the action of sheep pituitary extract.

Chemical Studies of the Augmenting Substance of the Sheep Serum

The euglobulin and pseudoglobulin fractions were removed from the experimental sheep serum by salting out with Na_2SO_4. For this purpose the serum was diluted with an equal volume of water and anhydrous Na_2SO_4

was added to make a 14.5% solution. The precipitated euglobulin was washed with 14.5% Na_2SO_4 solution and dissolved in water, enough salt being present in the precipitate to permit solution. The concentration of Na_2SO_4 in the supernatant solution and washings was then increased to 22% to precipitate the pseudoglobulin fraction. The data in table 8 show that the activity was carried down almost completely with the euglobulin precipitate. The pseudoglobulin fraction was only slightly active.

TABLE 9. ADSORPTION OF ENHANCING ACTIVITY OF SHEEP SERUM ON ALUMINA

Preparation no.	Type of alumina	Fraction tested	Equivalent of original serum	Deviation from controls	
				Ovaries	Uterus
			cc.	mg.	mg.
Euglobulin (*Sh1 S6E2*)	A	Untreated	1.5	+43.3	+54.4
		Eluate	1.5	+33.9	+67.0
Euglobulin (*Sh2 S12E*)	A	Untreated	1.5	+32.4	+33.6
		Eluate	1.5	+23.6	+35.7
Euglobulin (*Sh3 S13E*)	A	Untreated	1.5	+25.4	+59.5
		Eluate	1.5	+18.3	+63.4
Euglobulin (*Sh3 S6E*)	Brockmann	Untreated	3.0	+25.2	+37.9
		Eluate	3.0	− 3.4	−26.6
Serum (*Sh3 S4*)	A	Untreated	1.5	+17.9	+54.7
		Eluate	1.5	− 0.1	+ 8.9
		Filtrate	3.0	+12.7	+29.2
Euglobulin (*Sh3 S4E*)	A	Eluate	2.4	+25.0	+37.3
Serum (*Sh3 S4*)	Brockmann	Eluate	2.4	+ 4.2	+ 1.4
		Filtrate	2.7	+ 7.5	+50.9
Serum[1] (*Sh3 S4*)	A	Eluate	2.7	+ 0.5	− 0.9

[1] Diluted 10 times before treatment with alumina gel.

In this connection it is interesting to note that the augmentative activity of the serum of the horse and dog obtained by Thompson (9) by treating these animals with sheep pituitary extract was precipitated by $(NH_4)_2SO_4$ in the pseudoglobulin fraction. The observation was also made by this investigator that these sera alone produced follicle stimulating action in normal immature female rats. The results here reported may indicate a difference in the progonadotropic substances resulting from the chronic treatment with homozoic and heterozoic pituitary extracts.

In attempting to subfractionate the euglobulin fraction with Na_2SO_4 we found that about half of the activity appeared with the precipitate produced by a concentration of 12%. The remaining activity was distributed in the precipitates obtained between 12.5 and 14.5% Na_2SO_4.

The data in table 9 show that the active material may be readily adsorbed by alumina gel from the euglobulin fraction but not from whole serum. In carrying out the adsorption the serum or solution of the euglobulin fraction

was mixed with an equal volume of $\frac{0.2\ \text{M NaAc}}{0.2\ \text{M HAc}}$ buffer solution and treated two or three times with small amounts of the alumina preparations. The combined alumina was then extracted two or three times with 0.1 M Na_2HPO_4. The eluate was neutralized with dilute acid. By this treatment the activity is practically completely removed from solutions of the euglobulin fraction by Willstätter's type A alumina gel, but not by Brockmann's alumina. Neither alumina preparation, under the conditions of the experiment, removed the active substance from whole serum. A considerable portion of the activity remained unadsorbed in the filtrate.

Preliminary experiments with dialysis of the euglobulin fraction through Visking membranes indicate that reprecipitation of the euglobulins by this

TABLE 10. DIALYSIS OF EUGLOBULIN FRACTION CONTAINING THE ACTIVE SUBSTANCE OF SHEEP SERUM

Euglobulin preparation	Treatment	Equivalent of original serum	Deviation from controls	
			Ovaries	Uterus
		cc.	mg.	mg.
Sh1 S5E	Untreated	0.6	+28.6	+39.7
	Precipitate of 1st dialysis	1.68	+11.6	+15.3
	Supernatant of 1st dialysis	1.5	+15.6	+34.1
Sh1 S6E2	Untreated	1.5	+43.3	+54.4
	Supernatant of 1st dialysis	1.5	+37.1	+31.1
	Precipitate after dialyzing twice	1.2	+17.3	+20.0
	Supernatant of 2nd and 3rd dialysis	2.0	− 0.9	− 9.2
	Precipitate after dialyzing 3 times	1.5	+11.2	+12.7
	Precipitate after dialyzing 5 times	1.7	+ 7.5	+32.2
	Combined supernatant solutions	1.2	+ 8.0	+28.1

means does not carry down all of the activity (table 10). Dialysis for 96 hours against distilled water, which was changed frequently, until precipitation was complete, and continued dialysis of the supernatant solution for an additional 72 hours did not precipitate all of the active substance. Likewise dialysis of the precipitated material as many as 5 times by dissolving it in saline and continuing the dialysis until the precipitation was complete did not remove the activity from the precipitate. The supernatant after the first dialysis apparently contains little or no activity. It therefore appears that at least a part of the activity can be separated from the euglobulin precipitate.

Untreated serum and solutions of the euglobulin fraction in the frozen state retain their activity for a comparatively long time. One serum (*Sh1 S5*) showed little change in potency after remaining frozen for 10 months. Many preparations which have been reassayed after 2 to 3 months of such storage showed complete retention of activity.

Heating in a boiling water bath for 10 to 15 minutes renders the preparations completely inactive. However, it is possible that the active material may be adsorbed on the coagulated protein and may not be appreciably

absorbed following the injection of the suspension. Standing at pH 2 for 4 to 6 hours at 25° C. also causes inactivation. Here too, the active material may be adsorbed on the metaproteins which precipitate upon neutralization of the solution. In one instance in which the metaprotein precipitate was partially redissolved by making the solution somewhat alkaline, activity was demonstrable. Similar treatment at pH 11 produced little change in activity. In this case neutralization of the solution did not produce a precipitate.

Attempts to obtain active preparations in a dry, solid form have not

TABLE 11. INFLUENCE OF ACETONE AND ETHER ON AUGMENTATIVE ACTIVITY

Preparation used	Treatment	Whole serum or its equivalent	Deviation from controls		Remarks
			Ovaries	Uterus	
		cc.	mg.	mg.	
Sh1 S3 (serum)	Untreated	2.1	+16.9	+39.1	Saline extract was alkaline to phth. Neutralized with HCl
	10 vol. acetone. Ppt. extracted with saline	3.1	− 5.7	+ 0.4	
Sh1 S4 (serum)	Untreated	2.1	+28.9	+40.0	
	10 vol. acetone. Ppt. extracted with 0.05 N HCl	2.5	+12.2	+45.6	Ppt. dissolved almost completely
	Ether-soluble fraction of the serum	4.8	− 3.6	+ 0.6	Suspended in H_2O
	Serum after ether extraction	3.0	+19.8	+46.6	
Sh1 S5 (serum)	Untreated	2.1	+28.6	+40.5	
	50% acetone-soluble portion	2.0	+ 2.1	− 9.3	pH of serum adjusted to 7
	50% acetone-insoluble saline-soluble fraction	2.0	+ 5.4	+ 3.5	
	50% acetone-insoluble saline-insoluble fraction	2.0	+ 4.4	− 3.1	Suspended in H_2O
Sh1 S6E2 (euglobulin)	Untreated	1.5	+43.3	+54.4	
	Euglobulin ppt. washed with acetone then extracted with saline	1.5	+21.5	+25.5	
	Saline-insoluble fraction	2.1	+ 4.1	− 4.4	Suspended in H_2O
Sh3 S13E Ala (alumina eluate)	Untreated	1.5	+18.3	+63.4	
	44% acetone ppt. suspended in saline	1.5	+ 0.1	− 4.1	pH of eluate adjusted to 9
	50% acetone ppt. suspended in saline	1.5	− 6.3	− 4.8	
	50% acetone-soluble fraction	3.0	− 1.1	− 5.3	

been entirely successful. Whenever acetone is used the loss of activity is usually extensive (table 11). Here too, insoluble proteins result. In the case (*Sh1 S4*) in which the acetone-precipitated material almost completely dissolved in dilute hydrocholic acid, considerable activity was demonstrated. We have not yet attempted to repeat this treatment. Diethyl ether apparently does not affect the active material in the serum.

Desiccation of the euglobulin precipitate over $CaCl_2$ in a vacuum desiccator usually causes little loss of activity (table 12). Occasionally some inactivation is observed although the material remains completely soluble. We have not yet determined whether the activity is more stable in this form than it is in a frozen solution.

TABLE 12. VACUUM DESICCATION OF EUGLOBULIN PRECIPITATE

Serum and its euglobulin ppt.	Treatment	Equivalent of original serum	Deviation from controls	
			Ovaries	Uterus
		cc.	*mg.*	*mg.*
Whole serum	Untreated serum	3.0	+13.8	+44.4
Euglobulin ppt.	Not desiccated	3.0	+25.2	+37.9
Euglobulin ppt.	Desiccated	3.0	+15.7	+29.0
Whole serum	Untreated serum	1.5	+31.1	+41.7
Euglobulin ppt.	Not desiccated	1.5	+25.4	+59.5
Euglobulin ppt.	Desiccated	1.5	+ 6.9	+36.1
Whole serum	Untreated serum	1.5	+26.8	+58.0
Euglobulin ppt.	Not desiccated	1.5	+32.4	+33.6
Euglobulin ppt.	Desiccated	1.5	+31.1	+72.1
Whole serum	Untreated serum	1.5	+ 8.1	+39.2
Euglobulin ppt.	Desiccated	1.5	+16.6	+21.3
Whole serum	Untreated serum	3.0	+23.4	+59.9
Euglobulin ppt.	Desiccated	3.0	+ 8.7	+43.7

DISCUSSION

The results obtained with the serum of the sheep treated with sheep pituitary extract agree with our experiments on rats and add additional data showing that other animals treated with extracts of the pituitaries of the same species do not readily develop substances inhibitory to the injected material. This conclusion is supported by Rowlands (5) and Thompson (7) but not by Collip (4, 13) and Anderson and Evans (11). The fact that the testes of the ram (*Sh* 3) have been decreasing in size despite continued treatment with the sheep pituitary extract seems to indicate that insensitivity to the extract is developing in this animal. Nevertheless, we have been unable to detect inhibitory activity in either the whole serum or the globulin fractions. Although inhibitory activity has been reported (9, 12), as occurring in the pseudoglobulin fraction and we have found the augmentative activity concentrated mainly in the euglobulin fraction the possibility remains that the failure to detect an inhibitory substance is due to a masking effect. We are continuing the treatment of the sheep with this point in mind.

The experiments with the pigs treated with pig pituitary extract have as yet not given conclusive results. No augmentative activity was found in the sera of these animals. Apparently some inhibitory activity was present in some sera but this finding has not been consistent or typical. Then, too, the testes of the boar after a year of treatment were apparently normal. More data are needed before a definite conclusion can be made in regard to the reaction of pigs to a course of injections of pig pituitary extract.

The mode of action of the progonadotropic substance is not known. It does not seem likely that its effect is brought about by influencing the rates of absorption or excretion of the administered gonadotropic hormone since the augmentation is rather highly specific and is not produced with most gonadotropic preparations. It apparently does not exert its effect by influenc-

ing the hypophysis of the assay animals, since its administration alone does not produce a demonstrable effect. The active substance does not appear to have a luteinizing action by itself because it did not affect the ovaries of adult female rats or virgin rabbits and had little effect on the action of the follicle stimulating hormone of the urine of castrated women.

According to Evans et al. (14) and Bunde and Hellbaum (15) the relative inactivity of pituitary extracts in producing gonadal stimulation following their intraperitoneal administration is due to the presence of an antagonistic substance. In preliminary experiments we have found that the experimental sheep serum injected subcutaneously causes intraperitoneally injected sheep pituitary extract to produce a greater gonadotropic effect than the same quantity of the extract injected subcutaneously without serum. Possibly, this observation and our other experiments on the enhancing action of sera may be due to the antihormone or antibody to Evans' antagonistic substance as postulated by Thompson (9).

On the other hand, the precipitin reaction between the serum and the extract was negative in dilutions of 0 to 500 (only one sheep tested). Furthermore, the rather high specificity exhibited by the sheep serum does not seem to bear out the concept of an antibody to the antagonist since Evans found that the antagonistic substance prepared from the pituitaries of one species inhibited the response to extracts of the pituitaries of other species and of human pregnancy urine and pregnant mare serum. Obviously, more extensive data concerning both the inhibitory and augmentative phenomena must be obtained before a solution to this problem can be reached.

SUMMARY

The sera of sheep treated with chronic injection of sheep pituitary extract for as long as 262 days have not shown antigonadotropic activity. On the contrary, they continue to possess an enhancing action on the gonadotropic activity of this pituitary extract in immature female rats.

The active factor of these sera also augments the action of rat pituitary extract but not that of pig and beef pituitaries or the urine of pregnant and castrated women. When administered alone, it produces no apparent effect on the reproductive tracts of virgin female rabbits, immature or mature female rats.

The activity is almost completely salted out from the serum in the euglobulin fraction by Na_2SO_4. It is readily adsorbed from the solution of the euglobulin fraction, but not from whole serum, by Willstätter's type A alumina. It does not dialyze through Visking membrane.

The active material may be stored for long periods by freezing the serum or obtaining a dry powder of the euglobulin precipitate by vacuum desiccation over $CaCl_2$. Precipitation and desiccation with acetone, boiling and standing in acid solution (pH 2) have resulted in preparations in which activity usually could not be demonstrated. Treatment with diethyl ether or sodium hydroxide (pH 11 for several hours) has not materially affected the activity.

The serum is effective by subcutaneous or intraperitoneal administration. Intraperitoneally injected sheep pituitary extract in conjunction with subcutaneously injected sheep serum produces a greater gonadotropic effect than the same amount of extract administered subcutaneously without serum.

The sera obtained from two pigs and one sheep receiving a course of injections of pig pituitary extract have as yet failed to give conclusive results.

REFERENCES

1. Katzman, P. A., N. J. Wade and E. A. Doisy: *J. Biol. Chem.* 114: lvi. 1936.
2. Katzman, P. A., N. J. Wade and E. A. Doisy: *Endocrinology* 21: 1. 1937.
3. Katzman, P. A., N. J. Wade and E. A. Doisy: *Proc. Soc. Exper. Biol. & Med.* 38: 122. 1938.
4. Collip, J. B.: *Canad. M. A. J.* 36: 199. 1937.
5. Rowlands, I. W.: *Proc. Roy. Soc. B*, 124: 492. 1938.
6. Collip, J. B.: *Tr. A. Am. Physicians* 52: 130. 1937.
7. Thompson, K. W.: *Proc. Soc. Exper. Biol. & Med.* 35: 634. 1937.
8. Rowlands, I. W.: *Proc. Roy. Soc. B*, 124: 503. 1938.
9. Thompson, K. W.: *Proc. Soc. Exper. Biol. & Med.* 35: 640. 1937.
10. Spence, A. W., E. F. Scowen and I. W. Rowlands: *Brit. M. J.* 1: 66. 1938.
11. Anderson, E., and H. M. Evans: *Proc. Soc. Exper. Biol. & Med.* 38: 797. 1938.
12. Harington, C. R., and I. W. Rowlands: *Biochem. J.* 31: 2049. 1937.
13. Selye, H., J. B. Collip and D. L. Thompson: *Proc. Soc. Exper. Biol. & Med.* 31: 566. 1934.
14. Evans, H. M., K. Korpi, R. I. Pencharz and M. E. Simpson: *Univ. Calif. Publ. Anat.* 1: 237. 1936.
15. Bunde, C. A., and A. A. Hellbaum: *Am. J. Physiol.* 125: 290. 1939.

STUDIES ON THE INHIBITORY HORMONE OF THE TESTES[1]

III. HISTOLOGICAL EFFECTS ON THE MALE SEX ORGANS OF THE WHITE RAT

BEN VIDGOFF AND RICHARD HILL

From the Department of Pharmacology of the University of Oregon Medical School

PORTLAND, OREGON

THE PREPARATION of an extract from bull testes containing inhibitory properties has been reported in a previous publication (1). The effect of this extract on the weight of the male sex organs has been described (2). The present paper will be limited to a study of the effect on the histologic structure of the testicle, seminal vesicle and prostate of animals injected with this extract.

MATERIAL AND METHODS

The glands used for this study were those whose weight changes were previously reported (2). The normal histology of the rat prostate and seminal vesicle has been adequately described by C. R. Moore and his coworkers (3, 4). The division of the prostate referred to as the middle lobe by these authors is described by us as the lateral lobe. Since the lateral and posterior lobes are histologically similar, only the lateral lobe has been included in this report. The histology of the anterior lobe is not reported.

The tissues were fixed in formol-Zenker solution and stained with hematoxylin-eosin. The paraffin sections were cut at 10 micra.

Effect of the extract on the prostate. The most noticeable finding in the prostate is a shrinkage of the epithelial unit with a decrease in the diameter of the acini (fig. 1, 2). The interacinar connective tissue is much more abundant than in the control. It is cuboidal in most instances, but in some areas it is almost flat. There is no evidence of stratification. The cytoplasm stains more lightly than the normal, and the clear light area present in the normal epithelium between the nucleus and the lumen end of the cell is absent in the experimental animals. The nuclei are smaller, crowded and occupy a position in the center of the cell rather than in the basal region which is the normal condition. They show pyknotic changes and have lost the orderly arrangement seen in the control animals. In some areas the nuclei are not to be found in the cell. The lumina contain cell debris mixed with secretion. It is not possible to demonstrate any secretory changes in the cells with the stains used in this study.

Effect of the extract on the seminal vesicle. Here, again, the most noticeable change is a shrinkage of the epithelial unit and a marked increase in the intervesicular connective tissue (fig. 3, 4). The cell height is reduced and in some

[1] Aided by grants from the council on Scientific Research of the American Medical Association and Eli Lilly Co.

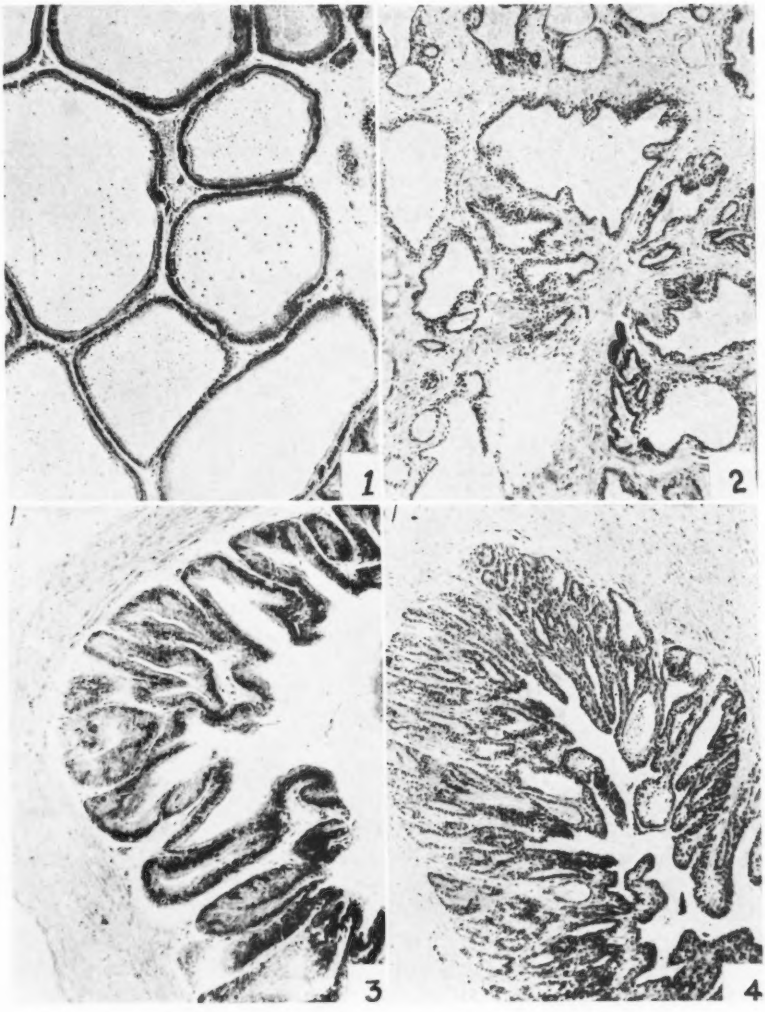


Fig. 1. PHOTOMICROGRAPH OF NORMAL RAT PROSTATE. Fig. 2. PROSTATE OF LITTER MATE WHICH RECEIVED EXTRACT. Fig. 3. SEMINAL VESICLE OF CONTROL (same animal as fig. 1). Fig. 4. SEMINAL VESICLE OF EXPERIMENTAL ANIMAL (same as fig. 2). (×84).

cases is but little more than the height of the nucleus. There are threads jutting from the epithelial cells into the lumen which is filled with debris. There is a decrease in the secretion visible in the lumina. The cytoplasm of the cell is lighter staining and vacuolated in places, usually at the base of the cell. The nuclei are darker staining, show pyknotic changes, and are centrally located.

Effect of the extract on the testicle. The effect of the extract on the testicle

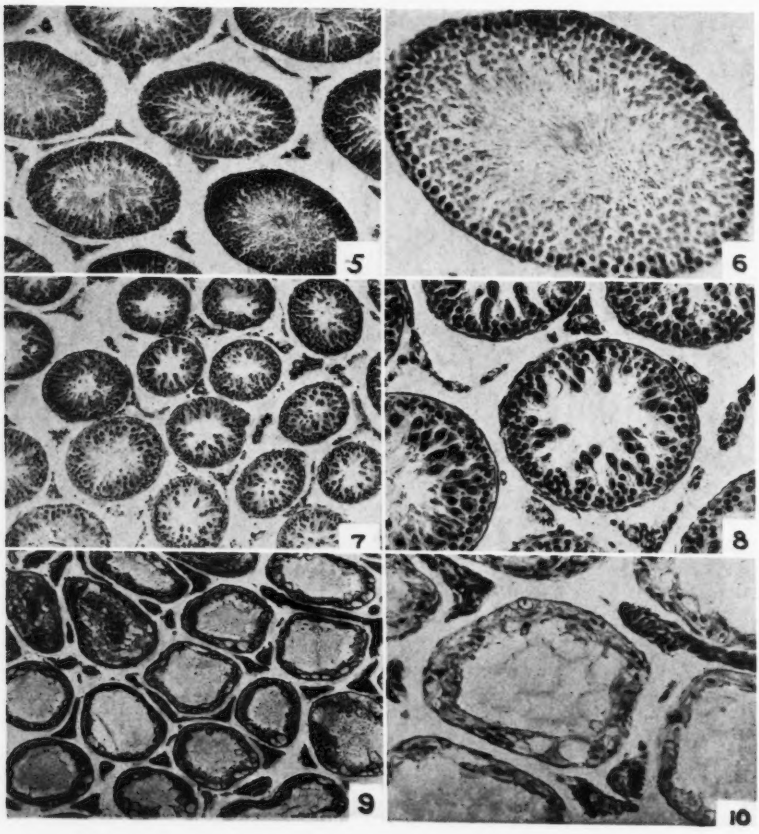


Fig. 5. PHOTOMICROGRAPH OF NORMAL RAT TESTICLE (same animal as fig. 1, 3). Fig. 7. TESTICLE OF EXPERIMENTAL RAT SHOWING SECOND STAGE CHANGES (same animal as fig. 2, 4). Fig. 9. TESTICLE SHOWING THIRD STAGE CHANGES IN THE EXPERIMENTAL RAT. (×72). Fig. 6, 8, 10. HIGHER MAGNIFICATION OF THE ABOVE FIGURES, EACH ONE CORRESPONDING TO THE FIGURE ON THE LEFT. (×172).

is not as constant as it is on the accessory organs. Three stages are seen. In the first stage, the changes are minimal and show reduction in the size of the seminiferous tubule, with some increase in the intertubular space, although no increase in the interstitial tissue can be demonstrated. There is no interference with the process of spermatogenesis except in a few dispersed small areas.

The second stage (fig. 7, 8) shows interference with the process of spermatogenesis. There is a marked decrease in the size of the tubule with increase in the intertubular space. Here again, no increase can be demonstrated in the interstitial tissue. Very few spermatozoa are to be seen and the process seems to be interrupted at the spermatid stage. The orderly arrangement of spermatogonia and spermatocytes seen as a characteristic of the normal animal are absent in the experimental animal. Their nuclei are smaller and pyk-

notic. The spermatid cells show cloudy swelling of the cytoplasm and indistinct cell margins. There are degenerative changes in the nuclei. The Sertoli cells seem to be intact in this stage except for some dispersed vacuolization. The lumina of the tubules are filled with debris.

The changes are maximal in the third stage (fig. 9, 10). There is an absence of spermatogenesis. Very few spermatogonia or spermatocytes are seen. The Sertoli cells form the border of the tubules which are markedly shrunken and, in some sections, free from any cellular or secretory debris. In others the lumina are filled with a solid mass of cellular debris. The Sertoli cells, which apparently withstand the degenerative effects better than the germinal cells, show in the cytoplasm, cloudy swelling, hydropic degeneration, absence of nuclei and marked vacuolization. In this stage the epididymi contain no spermatozoa. Some sections show all three stages. In others, either the first two or the last two stages appear together.

DISCUSSION

A comparison of these results with those on the weight of these organs shows that, in the main, the histologic changes parallel the decrease in weight of the organs.

The picture of the prostate and seminal vesicle, as revealed by this study, closely simulates the changes found in the castrated rat between the tenth and twentieth day after castration. The histologic picture of the testicle found in this study appears very similar to that found in the testicle of the hypophysectomized rat (5, 6).

Since these rats were neither castrated nor hypophysectomized, we offer the following as a tentative mechanism for the results produced: an inhibitory effect is produced on the anterior pituitary by the injection of this extract which interferes with or stops the production of male hormone in the testicle. This, in turn, produces the castration effects in the accessory sex organs.

SUMMARY

The effect of injections of an extract of bull testis possessing inhibitory action on the histologic structure of the sex organs of the male rat is shown.

The changes in the prostate and seminal vesicle are atrophic and degenerative in nature and simulate the changes seen in castrated rats.

The changes in the testicle are atrophic and degenerative in nature and are associated with decreased spermatogenic function.

The effects are discussed on the basis of the inhibitory action of the extract.

Until further study of the other endocrine organs, the mechanism of action suggested must be considered as tentative.

REFERENCES

1. Vidgoff, B., R. Hill and R. Kubin: To be published.
2. Vidgoff, B., R. Hill, H. Vehrs and R. Kubin: To be published.
3. Moore, C. R., D. Price and T. F. Gallagher: *Am. J. Anat.* 45: 71. 1930.
4. Moore, C. R., W. Hughes and T. F. Gallagher: *Am. J. Anat.* 45: 109. 1930.
5. Smith, P. E.: *Am. J. Anat.* 45: 205. 1930.
6. Crooke, A. C. and J. R. Gilmour: *J. Path. and Bact.* 47: 525. 1938.

ACTION OF TESTOSTERONE PROPIONATE ON THE STRUCTURE OF THE ANTERIOR PITUITARY OF THE FEMALE RAT WITH PARTICULAR REFERENCE TO THE EFFECTS OF PROLONGED ADMINISTRATION ON THE LEVELS OF CELLS[1]

J. M. WOLFE AND JAMES B. HAMILTON

From the Departments of Anatomy, Albany Medical College, Union University and Yale University Medical School

ALBANY, NEW YORK AND NEW HAVEN, CONNECTICUT

CONSIDERABLE information is now available concerning the action of male hormone substances on the structure of the anterior pituitary. Reese and McQueen-Williams (1) and Nelson and Gallagher (2) reported that injections of extracts containing male hormone into castrated rats prevented the post-castration increase in the size and number of the basophiles and their subsequent modification into castration cells. Nelson and Gallagher (3) later reported that injection of crystalline androstane-diol and androstene-dione prevented castration changes in the anterior pituitaries of female rats and induced the same type of degranulation that Nelson (4) had found to follow estrin administration. This would indicate that degranulation occurred both among the basophiles and eosinophiles (4). Androsterone was found to prevent castration changes (3). Wolfe and Hamilton (5) found that daily injections of 0.5 mg. of testosterone propionate in castrated immature female rats for a period of 10 days prevented the usual castration changes in the basophiles, a majority but not all of the basophiles in the gland being either completely or partially degranulated. No constant changes were noted in the eosinophiles, which occurred at practically the same levels in the injected and control rats. The pituitaries of the injected rats were not increased in weight, in fact they weighed slightly less than those of the control rats. Similar experiments were carried out in normal immature rats (6) with similar results. Allanson (7) tested the capacity of a number of androgens to maintain the normal structure of the anterior lobe following castration. The effectiveness of these substances in this respect was correlated with their capacity to maintain the accessory organs. In a later paper Nelson and Merckel (8) reported degranulation of the basophiles following administration of testosterone, androstane-dione and androstene-dione in normal

[1] These studies were aided by a grant from the International Cancer Research Foundation.

female rats. The first two substances also induced an increase in the size of the corpora lutea. Wolfe and Hamilton (9) found that injection of testosterone propionate induced luteinization in the ovaries of normal female rats. In the pituitaries degranulation of the basophiles was found and in some degranulation in considerable numbers of the eosinophiles along with a reduction in the relative numbers of these cells were also noted. However, it is doubtful if these particular experiments definitely indicate that injection of male hormone induced degranulation in the eosinophiles because they were carried out on female rats somewhat older than those which were used as controls. We have recently found that advancing age is associated with a decrease in the levels of the eosinophiles and that degranulated forms may be fairly abundant even in normal females (10, 11). We feel, therefore, that these studies should be repeated using experimental and control rats of the same age. Mazer and Mazer (12), in a very recent paper, concerned with prolonged treatment of immature and mature female rats with testosterone propionate, found that in the immature rats the pituitaries, the adrenals and the ovaries were reduced in weight, whereas in mature animals only the adrenals and the ovaries presented significant decreases in weight.

Examination of the available data as a whole indicates that administration of androgens does not increase the weight of the pituitary. There is general agreement that injection of these substances prevents castration changes in the pituitary and induces degranulation of the basophiles. In only two instances has degranulation of the eosinophiles been reported. In this paper the results of two series of experiments are presented. In series one we have studied the changes in the anterior lobe which resulted from injections of large amounts of testosterone propionate into immature female rats. These animals were divided into Groups A and B. In series two, the action of prolonged injection of the same substance on the anterior pituitary of the mature female rat was studied.

MATERIALS AND METHODS

The majority of the pituitaries were fixed in Regaud's fluid, stained by a modification of Mallory's triple stain (13), and were cut serially. Such preparations were used for general histologic study and cell counts. The results of these counts and other quantitative data were analyzed statistically. Although this staining method gives excellent cellular differentiation and discloses a certain degree of cytologic detail, it does not differentiate between the eosinophilic granules and the mitochondria. Methods making this differentiation possible were first described by Severinghaus (14). In this study somewhat similar cytologic methods were applied to some of the pituitaries. This material was fixed in Champy's fluid, dehydrated and embedded according to the procedures described by Severinghaus (14) and in a personal communication. A majority of the material was chromated following fixation by the modified Champy-Kull method of Severinghaus. A lesser amount of tissue was osmicated (Nassonov-Kolatchev technic). All tissue was cut serially at 3 micra. In these studies the chromated tissue was used for general cytologic study except for the Golgi apparatus. We have used both the staining methods of Severinghaus (14) and Volkonsky (15) during the course of this work but in most instances a modified

procedure involving the use of Altmann's acid fuchsin, orange-G and Goodpasture's polychrome methylene blue, was used (16). In sections stained by this method the mitochondria were stained a brilliant fuchsin, the eosinophilic granules an orange-red, and the basophiles deep blue. If a less intense fuchsin stain was applied, the mitochondria took a lighter fuchsin and the eosinophilic granules stained yellow-orange. The ovaries and accessory tissue were fixed in Bouin's fluid and stained in hematoxylin and eosin. The ovaries were cut serially while representative sections of the uteri and vaginae were cut.

RESULTS

Series 1, Group A. Twenty-two immature female rats, 25 to 35 days of age, received daily injections of from 2 to 8 mg. of testosterone-propionate[2] in peanut oil either for 10 or for 20 days. Two mg. of the androgen was dissolved in 0.1 cc. of oil. Since the results obtained with these various doses were essentially the same, all animals were considered in a single group. Sixteen litter-mate sisters were used as controls. The average pituitary weight of the experimental rats was slightly less than that of the control rats (table 1). In individual litters the pituitary weights of the experimental rats were either slightly less or the same as those of the controls, never

TABLE 1. A SUMMARY OF QUANTITATIVE DATA INDICATING THE EFFECTS OF INJECTION OF ANDROGEN INTO IMMATURE FEMALE RATS

	No. rats	Body wt.	Pituitary wt.	Ovary wt.	Levels of cell types				Mitoses per section
					Eos.	G. Basos.	N.G. Basos.	Chrom.	
Testosterone propionate	22	99	3.8	11.7	34.5	0.5	5.5	59.5	Chrom. 5.4 Eos. 1.7
Control	16	98	4.4	20.1	35.2	5.2	2.5	57.1	Chrom. 9.2 Eos. 3.0

greater. The average ovarian weight in the control rats was 20.1 mg., in the injected rats 11.7 mg.

The pituitaries of these rats were fixed in Regaud's fluid and prepared for general histologic study. Histologically, in the anterior pituitaries of the injected rats degranulation of the basophiles was almost complete in most instances, but entirely so in only 7 rats. The non-granular basophiles were normal in size and had varying amounts of light blue cytoplasm. The negative image of the Golgi apparatus was visible in many instances and showed no evidence of hypertrophy. The eosinophiles presented no appreciable alterations. Cell counts showed that the levels of these cells were the same in both the experimental and control groups (table 1). In both groups the negative image of the Golgi body was seen closely applied to the nucleus in many cells. Small numbers of cells in which the Golgi apparatus was quite prominent and in which there appeared to be a slight reduction of granules were noted in both groups. Chromophobes were slightly more abundant in

[2] Testosterone propionate was furnished by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

the injected rats, probably because some of the degranulated basophiles had passed into these cells. Three representative slides from each gland were examined carefully for mitotic figures. In table 1 these are expressed as the number counted per section. Mitotic figures were considerably more abundant in the controls rats, both in the eosinophiles and the chromophobes. The difference was not great enough, however, to indicate that injection of male hormone resulted in an inhibition of mitotic division. Thus, findings obtained by this particular technic indicated that the cellular reaction to administration of massive amounts of male hormone substance for a short period was degranulation in the basophiles and a reduction of the number of the granular basophiles in the gland (table 1).

Series 1, Group B. Six immature, 25-day-old, litter-mate female rats were castrated. Three of these received daily injections of 2 mg. of testosterone propionate in 0.1 cc. of peanut oil for 15 days; the remaining three served as controls. At autopsy, 24 hours after the final injection, the pituitaries were divided sagitally into two portions and prepared for cytologic study. One half of each gland was chromated and stained for general cytologic study, the remaining half osmicated for Golgi studies.

The eosinophiles in the anterior pituitaries of the injected and the castrated control rats were similar both in appearance and in abundance. A majority of these cells were small or medium in size but a few larger cells were seen. In general, they were well filled with granules but in both the injected and the control rats occasional eosinophiles were seen in which the granular content was greatly reduced. In appreciable numbers of cells in the non-osmicated tissue the negative image of the Golgi apparatus was seen closely applied to the nucleus. In a few of the medium and the larger cells the Golgi region was quite prominent and there was often some reduction of granules in this region. Large, brilliantly stained mitochondria were found scattered among the eosinophilic granules. In the smaller cells only a few were seen, often lying close to the nucleus. In the larger cells they were more abundant and in many cells they showed some tendency to be concentrated in the Golgi region. Study of the osmicated material in general confirmed the observations of Severinghaus (17) who found that the Golgi apparatus in the eosinophiles and basophiles differed in form and location in the cell and that in the chromophobes the Golgi apparatus was either of the eosinophilic or the basophilic type. In the eosinophiles the Golgi apparatus in most instances appeared as a dense network, somewhat semilunar in form, closely applied to the nucleus. No noteworthy difference was observed between the eosinophiles of the experimental and control rats as far as the Golgi apparatus was concerned.

The basophiles in the injected and control rats differed markedly both in appearance and in number. In the controls they were increased in size and number typical of 15-day immature castrates. A majority of the cells were quite large and took a basophilic stain which varied somewhat in intensity in the different cells. Numerous cells appeared to have deep blue granules of a flocculent type while others had a more homogeneous cyto-

plasm which was sometimes finely vacuolated. The latter cells were usually less intensely basophilic and the cytoplasmic material in the vacuoles took a lighter stain than the intervacuolar strands of cytoplasm. In the non-osmicated sections the Golgi apparatus most often appeared as the negative image but in many instances it stained blue, a finding previously noted by Kirkman (18) in the guinea pig. In the osmicated sections the Golgi apparatus was roughly circular in shape and lay free in the cytoplasm between the nucleus and the cell membrane. Irregular osmicated strands often passed through the center of the apparatus from one wall to the other. In general, the Golgi apparatus was much larger in the basophiles of the control rats than in those of the injected animals. Mitochondria were abundant in most of the basophiles especially in the Golgi region. The excentric nucleus was basophilic and had a prominent nuclear membrane. One or two nucleoli were usually seen.

In the injected rats the basophiles were almost completely degranulated and were not abundant. Most were medium in size with scant amounts of fragmentary light blue cytoplasm. The nucleus was usually rather basophilic. In non-osmicated sections the Golgi apparatus either appeared as the negative image or the entire Golgi region was stained a dull blue. In the osmicated sections it was found that the Golgi apparatus was smaller and more compact and that the osmicated strands making up the network were thicker or wider than they were in the larger basophiles of the control rats. Brilliantly stained mitochondria were usually abundant.

Series 2. It was demonstrated by McQueen-Williams (19) and by Wolfe and Ellison (20) that histologic differences exist between the anterior pituitaries of male and female rats. In males the incidence of both the basophiles and the eosinophiles is higher than in the female, and both types tend to be larger and better filled with granules. A few typical signet-ring castration cells are found in the anterior lobes of many normal male rats (20). Later Pfeiffer (21) demonstrated that the cellular pattern of the anterior pituitaries of male and female rats is at least to a great extent governed by the gonads. He castrated male rats at birth and implanted an ovary into the cervical region. Some rats received a second ovarian implant in the anterior chamber of the eye at puberty. Both follicular growth and the production of corpora lutea occurred in the implants. Histologic study of the anterior pituitaries of these animals revealed a cellular pattern typical of that of a female rat. Likewise, it was found that castration of female rats at birth and subsequent implantation of testes resulted in a pituitary similar in structure to that of a male.

The experiments described below indicate that injection of testosterone propionate into female rats, beginning at about 60 days of age, results in a cellular pattern in the anterior lobe which closely approaches that of the male. One or two females from a litter of rats received daily androgen for 90 days. For each injected female an untreated litter-mate sister and brother were used as controls. At the end of the injection period the anterior pituitary of the injected female rat was compared histologically with those of its

untreated brother and sister to ascertain which it more nearly resembled. Four doses of testosterone propionate were used in different groups of rats: 1 mg., 0.5 mg., 0.25 mg., and 0.1 mg. in 0.1 cc. of peanut oil, all daily for 90 days. In each group there were 4 to 6 injected females and equal numbers of control brothers and sisters.

It was found that with any of these 4 doses the injected females weighed somewhat more at the end of the experiment than their control sisters but considerably less than their control brothers (table 2). McEwen, Selye and Collip (22) have previously reported that injection of testosterone in normal female rats appeared to give slight growth stimulation. Since the 3 groups differed in body weight, the figures in table 2 represent milligrams of pituitary weight for 100 grams of body weight. On this basis the pituitary weights in the injected females were much more comparable to those of their untreated brothers than those of their untreated sisters. It is known that the adrenals of female rats are relatively heavier than those of males (23), a finding which our data support. In table 2 the weights of the adrenals in the 3 groups are expressed in the same terms as the pituitary weights. Our data indicate that the adrenal weights were decreased in the injected females and that the decrease was proportional to the amounts of the androgen injected. In rats receiving 1 mg. daily the adrenals were slightly less in weight (per unit of body weight) than were those of the control males and much less than those of the control females. In the progressively smaller doses the adrenals of the injected females approached those of their control sisters in weight and diverged from those of their brothers. Since the pituitary weights in the injected females were restricted at all 4 injection levels, our findings would suggest a differential capacity of androgen to restrict the weights of the pituitary and of the adrenal in the rat.

In table 3 data on all 4 groups of experimental rats are grouped together and analyzed statistically.[3] Data on the females and males are handled similarly. The differences between the adrenal and pituitary weights in the injected females and those of the control females were highly significant but the differences between these organs in the injected females and their control brothers were not statistically significant (table 3). On the other hand, the body weights of the injected females were statistically closer to those of their control sisters.

Cell counts were made on all pituitary sections stained by the modified Mallory method. Quantitative data on the different groups are summarized in table 2, and are analyzed statistically in table 3. Histologically and quantitatively, the anterior lobes of the injected females were quite similar to those of their control brothers but differed markedly from those of the control sisters (tables 2 and 3). The higher doses of the androgen were more effective in inducing these changes than were the lower (table 2).

In the injected females and the control males the eosinophiles were very abundant and usually well filled with granules. In many the negative image of the Golgi was seen lying closely applied to the nucleus. In some cells in

[3] We are indebted to Dr. Ethel Burack for the statistical analysis of this material.

both groups the Golgi apparatus was larger and more prominent; in some of these cells the granular content was slightly reduced. As a whole, however, there was no evidence of any appreciable degranulation among the eosinophiles in either group. In the control females these cells generally were somewhat smaller. In many cells the negative image of the Golgi apparatus was seen and in some cells it was quite prominent. In a moderate number of the latter cells there seemed to be some reduction in the granular material.

In the injected females many large granular basophiles and even a few typical signet-ring castration cells were found. The levels of these basophilic elements closely approached the levels found in the control males, particu-

TABLE 2. A SUMMARY OF QUANTITATIVE DATA INDICATING THE EFFECTS OF CHRONIC ADMINISTRATION OF ANDROGEN AT 4 INJECTION LEVELS

Treatment and sex	No. rats	Average wt.	Ovary wt.	Testis wt.	Pit. wt. per 100 gm.	Adr. wt. per 100 gm.	Eos.	Gran. bas.	N. G. bas.	Castration cells	Chrom.
			mg.	*gm.*							
Control ♂	6	372	—	3.2	2.3	12.5	49.0	6.1	0.6	.9	43.4
1000 γ T P daily ♀	6	252	13.8	—	3.1	11.7	47.3	5.2	1.8	.6	45.1
Control ♀	6	224	52.6	—	4.6	21.4	31.8	1.8	3.0	—	63.4
Control ♂	5	368	—	3.0	2.4	11.1	47.0	5.1	1.1	.4	46.4
500 γ T P daily ♀	4	288	22.0	—	2.8	12.9	46.0	3.5	0.3	+	50.2
Control ♀	4	257	76.0	—	4.3	19.5	29.6	2.2	2.1	—	66.1
Control ♂	5	418	—	3.2	2.5	9.2	46.8	4.5	1.7	.9	46.1
250 γ T P daily ♀	5	300	25.0	—	3.3	15.7	40.2	1.9	0.4	+	57.5
Control ♀	5	255	70.0	—	4.9	20.0	32.2	2.0	1.6	—	64.2
Control ♂	5	397	—	3.2	2.5	12.2	51.0	3.4	0.8	.6	44.2
100 γ T P daily ♀	5	277	28.0	—	3.2	17.0	41.8	2.7	2.0	+	53.5
Control ♀	5	234	50.0	—	4.7	21.6	31.8	2.8	2.8	—	62.6

larly in the animals receiving the higher doses of androgen (table 2). This came as a distinct surprise since all previous studies had indicated that injection of male hormone induced degranulation of the basophiles. It should be pointed out that in previous experiments much shorter injection periods were used and furthermore it should be remembered that the hypophysis-gonad balance in the normal male is such that a fairly high level of granular basophiles and even a few castration cells may normally exist. In the females receiving the 1 mg. level of androgen the basophilia closely approached that found in normal males but in rats receiving smaller doses, the levels of the basophiles were lower although occasional castration cells were seen. Our data would indicate that 1 mg. daily of testosterone propionate sufficed to induce a basophilic pattern in the female similar to that of the males but would suggest that smaller amounts may influence the eosinophilic pattern toward the condition found in the male.

In table 3 the cell counts from all groups with all 4 doses of androgens were combined and compared statistically with those obtained from the control female and male groups. It is seen that whereas the level of the eosinophiles is significantly different between injected females and control males,

TABLE 3. A STATISTICAL ANALYSIS OF QUANTITATIVE DATA INDICATING EFFECTS OF INJECTION OF ANDROGEN IN THE VARIOUS GROUPS OF RATS

Observations	Control females			Diff. between means	Females injected with testosterone propionate			Diff. tween means	Control males		
	No. observations	Mean+P.E.$_m$	S.D.+P.E.$_\sigma$	P.E. Diff.	No. observations	Mean±P.E.$_m$	S.D.±P.E.$_\sigma$	P.E. Diff.	No. observations	Mean±P.E.$_m$	S.D.±P.E.$_\sigma$
Eosinophiles	15	31.4±0.37	2.04±0.26	14.9	17	44.2±0.78	4.65±0.55	3.8	18	48.3±0.74	4.49±0.52
Granular basophiles	15	2.1±0.21	1.18±0.15	4.3	17	3.5±0.25	1.46±0.17	4.5	18	5.0±0.22	1.34±0.15
Non-gran. basophiles	15	2.3±0.17	0.93±0.12	4.2	16	1.2±0.20	1.14±0.14	0.4	17	1.1±0.11	0.68±0.08
Castration cells					16	0.23			17	0.8+0.07	0.46±0.05
Chromophobes	15	64.2±0.43	2.39±0.31	23.3	17	51.6±0.34	2.01±0.23	8.4	18	45.0±0.71	4.31±0.50
Pituitary weight	20	11.4±0.25	1.61±0.18	7.8	20	8.6±0.26	1.70±0.19	2.8	21	9.7±0.29	1.93±0.21
Weight of adrenals	20	51.6±1.12	7.23±0.79	8.0	20	37.3±1.44	9.30±1.01	2.8	21	42.6±1.24	8.22±0.88
Weight of ovaries	20	63.6±1.76	11.36±1.24	19.2	20	21.8±1.28	8.26±0.90				
Weight of testes									21	3.0±0.10	0.66±0.07
Weight of prostate & seminal vesicles									15	2.2±0.13	0.70±0.09
Weight of animals	20	240 ±3	21 ±2	6.4	20	277 ±5	34 ±4	9.2	21	380 ±10	65 ±7

Standard Deviation was calculated by formula $\sigma = \sqrt{\frac{\sum(x)^2}{n-1}}$

Ratio of difference between means to probable error of difference was calculated by formula $\frac{M_1 - M_2}{\sqrt{(P.E._{m_1})^2 + (P.E._{m_2})^2}}$. When this ratio is 3 or over, it is considered statistically significant.

this difference is not nearly so great as that which occurred between the levels of these cells in the injected females and the control females. In considering the granular basophiles it is found that the levels of these cells in the injected females were statistically slightly closer to the levels of these cells in the control females (table 3). This was undoubtedly due to the lowered response of these cells in the experimental groups receiving the lower amounts of the androgen. This finding strongly suggests the possibility that the eosinophiles respond to lower dosages of androgen than do the basophiles. Further study of table 3 indicates that the levels of the chromophobes in the injected females approximated the levels found in the control males and diverged greatly from the levels found in the control females.

In rats of this series which received the 1 mg. dose of androgen the pituitaries were divided sagitally at autopsy. One-half was fixed in Regaud's fluid and stained by the modified Mallory method. This material was used in the studies involving cell counts which were just described. The remaining half was fixed in Champy's fluid and prepared for cytologic study. Osmicated preparations were not made. Tissue from 18 rats was handled by this procedure which made it possible to study sections from the same gland by two different methods, one of which was valuable for general histologic study and for cell counts and the other which was essential for study of finer cytologic detail.

The eosinophiles in the injected females and the control males were quite similar in appearance. Generally they tended to be large and well filled with granules. In many cells in both groups the negative image of the Golgi was visible and in some instances was quite prominent. In a few cells in glands from both groups of rats there was a considerable reduction in granular content although there was no evidence of any considerable degranulation. Large brilliantly stained mitochondria were seen which were usually more abundant in the larger cells and possibly even more so in cells in which there was some reduction of granules. In the control females the eosinophiles were less abundant and generally somewhat smaller than in the other 2 groups. There were perhaps more cells which showed evidence of loss of granules, but the differences between the 3 groups in this respect were not marked.

The basophiles in the injected females and the control males were also similar in appearance although in any one gland the basophiles varied in appearance and although the degree of basophilia was not marked in rats receiving the lower levels of androgen. In all glands large cells with deeply staining flocculent granules were found. The nucleus was excentric and basophilic. The Golgi apparatus appeared either as the negative image or was stained positively with blue. Large brilliant mitochondria were fairly abundant. Other cells were seen in which there was an apparently homogeneous light blue cytoplasm which in some instances was considerably broken up and fragmentary. The Golgi apparatus usually appeared as the negative image. Mitochondria were abundant, sometimes slightly more so than in the granular cells. A few cells were seen in which the cytoplasm was

finely vacuolated. This process might involve a cell completely or only partially. Mitochondria were not generally seen in the vacuolated portions of a cell. In some glands, generally those in which basophiles were most abundant, some basophiles were seen in which the cytoplasm contained many clear vacuoles, giving the cell somewhat of a sieve-like appearance. These vacuoles were never very large but the number varied from cell to cell. The Golgi apparatus usually appeared as the negative image and they contained moderate numbers of large prominent mitochondria. These cells were usually most abundant on the periphery of the gland. In the control females, basophiles were less abundant than in the other 2 groups. Practically all of these were either the large deeply staining granular cells or the lighter staining cells in which granules were not observed. The latter were relatively more abundant in this group than in the injected females or the control males.

The chromophobes were generally quite small in all groups. In most instances the cell membranes were definite. The amount of cytoplasm was scant and usually stained blue. Mitochondria were seen in practically all cells and in a few were quite abundant.

Our findings indicate, therefore, that the anterior lobes of the injected females and the control males were cytologically quite similar to each other and more or less distinct from those of the control females. In the control females there were many degranulated basophiles, indicating a secretory activity in these cells, whereas in the injected females and the control males there was less evidence of secretion and considerable evidence of storage. There was relatively little evidence of degranulation among the eosinophiles in any of the groups, but as a whole, there was most in the control females.

DISCUSSION

The findings reported in this paper support the original observations of Pfeiffer who found that the gonads exerted a definite influence on the cellular pattern of the anterior pituitary. His results along with our own then explain the earlier reports which indicated that the cellular patterns in the anterior lobes of male and female rats are distinct. This distinct difference in pituitary histology between the two sexes does not exist in all species. Kirkman (18) found no significant sex difference in the guinea pig, an observation which we have confirmed. In the human species, Rasmussen (24) found that basophiles were more abundant in men but that there were no differences in the levels of the eosinophiles. Such a species difference in the anterior pituitary in the matter of distinct cellular patterns in male and female is only one of the species differences known to exist in this gland.

Both in Pfeiffer's studies and in ours, experimental treatment was initiated when the animals were either immature or still quite young, so we have no evidence that a cellular pattern in the anterior lobe, already definitely established as a female type, was changed over to a male type. At present, the exact time of which the mature cellular pattern is established in the rat is not definitely known. Severinghaus (25) found that in 20-day-old

rats large basophiles were more abundant in the female pituitary than in the male. This is in contrast to the condition in fully mature animals where both basophiles and eosinophiles are more abundant in the male gland. These findings indicate that a reversal, at least as far as the basophiles are concerned, in the anterior lobe cellular pattern in the two sexes occurs between 20 days of age and maturity. These findings correlate well with those of Clark (26) who has described such a reversal in the gonadotropic hormone content of the anterior lobes of male and female rats. At 20 days of age the female pituitary contains more gonadotropic hormone than that of the male. As the animals approach puberty and become mature, the male pituitary gradually becomes more effective in stimulating the ovaries of test animals. We feel that the mature cellular patterns in the two sexes are formed during the period the animal is approaching maturity and possibly during early maturity. There is a definite need for histologic studies on the pituitaries of male and female rats of these ages.

The findings of fairly large numbers of granular basophiles and even a few castration cells in the pituitaries of female rats receiving androgen was not entirely expected since it seems fairly well established that injection of androgen in short-time experiments will degranulate the basophiles. Furthermore, the prevention of both a basophilia and the formation of castration cells in gonadectomized rats has commonly been accepted as a criterion of effectiveness of androgen. Since all available evidence indicates that in our chronic experiments the initial pituitary reaction was one of basophilic degranulation, we must assume that after this initial reaction there was an increase in the number and size of the granular basophiles and the formation of a few castration cells even though injections were continued. It is noteworthy that such a reaction was most pronounced in the rats receiving the highest levels of androgens. However, since in normal male rats appreciable numbers of granular basophiles and a few castration cells are compatible with the levels of androgen necessary in the system to maintain the accessories, it is not irrational to assume that the same should occur in females which received considerable amounts of androgen.

We failed to find any evidence in either the acute or chronic experiments that injection of male hormone induced degranulation among the eosinophiles or reduced the relative numbers of those cells found in the gland. In fact, injection of androgen materially increased the levels of these cells in the prolonged injection period. This would offer at least a partial explanation for the high level of these cells in the male rat while the much lower levels in females are almost certainly due to the more or less continuous action of estrogen, now generally recognized to be capable of degranulating the eosinophiles and reducing their levels in the gland. The fact that androgens are compatible with a high level of eosinophiles would probably also explain the earlier results of Severinghaus (17) and Ellison and Wolfe (26), who found that castration in male rats resulted in decreased levels of these cells.

It seems evident that the reaction of the anterior lobe to androgen and estrogen differs in several details. Estrogens induce a hypertrophy of the

gland and degranulation in both chromophilic types, which is accompanied by a hypertrophy of the Golgi apparatus. There is an increase in the size and number of the chromophobes in which the Golgi apparatus may be very large. As pointed out by Severinghaus (25, 28) these are not ordinary chromophobes but are active cells, releasing secretion as rapidly as it is formed, and that the whole process is one of activation. In prolonged experiments an intensification of the above reaction occurs which may eventually lead to adenomatous changes (29, 30, 31). Most evidence points to the fact that injection of androgen does not induce pituitary hypertrophy. In the acute experiments reported in this paper, basophilic degranulation was found but the Golgi apparatus was not enlarged. Degranulation among the eosinophiles was not found in either the acute or chronic experiments and in the latter these cells were actually increased in androgen-injected rats. Also, the degranulation of the basophiles found in the acute experiments was not intensified in the chronically injected rats; rather, there was evidence of an actual storage of granular material in the cell. These observations emphasize the fact that at the present time our knowledge as to how the anterior lobe reacts to androgen is largely incomplete, and indicate the need for further studies, both short-time and chronic, involving several types of test rats. These findings confirm the earlier observations of Hamilton (32) who found that testosterone propionate, injected into females, exerted a definite masculinizing effect.

SUMMARY

Immature female rats received daily injections of from 2 to 8 mg. of testosterone propionate daily for periods of 10 or 20 days. Litter-mate sisters were used as controls. The average pituitary weight of the experimental rats was slightly less than that of the controls. It was found that in the injected rats degranulation among the basophiles occurred but noteworthy changes were not observed in the eosinophiles.

In a second series female rats received chronic administration of androgen daily for 90 days. Four levels of injection were used: 1 mg., 0.5 mg., 0.25 mg., and 0.1 mg. Litter-mate sisters and brothers were used as controls. Judged in relation to body weight, the weight of the pituitaries and the adrenals of the injected females were more comparable to those of their untreated brothers than to those of their untreated sisters. An increase in the levels of the eosinophiles and granular basophiles occurred in the anterior pituitaries of the injected females resulting in a cellular pattern in this gland which closely approached that of their untreated brothers and widely diverged from that of the control sisters. This masculinizing effect was most prominent in the females receiving the highest amounts of androgen.

Cell counts were made on the pituitary sections and the quantitative data were handled by statistical methods.

REFERENCES

1. Reese, J. B., and M. McQueen-Williams: *Am. J. Physiol.* 101: 239. 1932.
2. Nelson, W. O., and T. F. Gallagher: *Anat. Rec.* 64: 129. 1935.
3. Nelson, W. O., and T. F. Gallagher: *Science* 84: 230. 1936.

4. Nelson, W. O.: *Proc. Soc. Exper. Biol. & Med.* 32: 452. 1934.
5. Wolfe, J. M., and J. B. Hamilton: *Proc. Soc. Exper. Biol. & Med.* 36: 307. 1937.
6. Wolfe, J. M., and J. B. Hamilton: *Endocrinology* 21: 603. 1937.
7. Allanson, M.: *Proc. Roy. Soc. London s.B.* 125: 196. 1937.
8. Nelson, W. O., and C. G. Merckel: *Proc. Soc. Exper. Biol. & Med.* 36: 823. 1937.
9. Wolfe, J. M., and J. B. Hamilton: *Proc. Soc. Exper. Biol. & Med.* 37: 189. 1937.
10. Wolfe, J. M., W. R. Bryan and A. W. Wright: *Am. J. Cancer* 34: 352. 1938.
11. Wolfe, J. M.: Unpublished data.
12. Mazer, M., and C. Mazer: *Endocrinology* 24: 175. 1939.
13. Cleveland, R., and J. M. Wolfe: *Anat. Rec.* 51: 409. 1932.
14. Severinghaus, A. E.: *Anat. Rec.* 53: 1. 1932.
15. Volkonsky, M.: *Bull. d'Histol. Appl.* 3: 220. 1928.
16. Wolfe, J. M., and W. R. Fain: *Stain Technol.* In press.
17. Severinghaus, A. E.: *Anat. Rec.* 57: 149. 1933.
18. Kirkman, H.: *Am. J. Anat.* 61: 233. 1937.
19. McQueen-Williams, M.: *Anat. Rec. suppl.* 58: 78. 1934.
20. Wolfe, J. M., and E. T. Ellison: *Anat. Rec. suppl.* 58: 93. 1934.
21. Pfeiffer, C. A.: *Endocrinology* 21: 812. 1937.
22. Donaldson, H. H.: The Rat: Data and reference tables. Wistar Institute, 1924.
23. McEwen, C. S., H. Selye and J. B. Collip *Proc. Soc. Exper. Biol. & Med.* 36: 390. 1937.
24. Rasmussen, A. T.: *Proc. Assoc. Res. Nervous and Mental Dis.* 17: 118. 1936.
25. Severinghaus, A. E.: Sex and Internal Secretions. Williams and Wilkins, 1939.
26. Clark, H. M.: *Anat. Rec.* 61: 175. 1935.
27. Ellison, E. T., and J. M. Wolfe: *Endocrinology* 18: 555. 1934.
28. Severinghaus, A. E.: *Physiol. Rev.* 17: 556. 1937.
29. Cramer, W., and E. S. Horning: *Lancet* 1: 247. 1936.
30. McEwen, C. S., H. Selye and J. B. Collip: *Lancet* 1: 775. 1936.
31. Wolfe, J. M., and A. W. Wright: *Endocrinology* 23: 200. 1938.
32. Hamilton, J. B.: *Anat. Rec. suppl.* 67: 22. 1937.

A COMPARISON OF THE EFFECTS OF MALE AND FEMALE SEX HORMONES ON IMMATURE FEMALE RATS

FRED W. CLAUSEN AND CLAY B. FREUDENBERGER
From the Department of Anatomy, University of Utah
SALT LAKE CITY, UTAH

ONE GAINS from the literature the impression of a possible antagonistic action between theelin and testosterone in certain instances. In mature ovariectomized mice Robson (1) found that the simultaneous administration of testosterone with estrone inhibited the estrous reaction of the vagina which was produced by estrone alone. Korenchevsky, Dennison and Hall (2) stated that the suppression of the cornifying effects of estrone, or of estradiol, by testosterone propionate may be considered as an antagonistic action in the rat. Wolfe and Hamilton (3) reported that the hypophyseal enlargement and basophilic degranulation resulting from estrone therapy in the rat were negated by the addition of testosterone acetate. An inhibition of estrogenic effects on the skeleton of mice by testosterone propionate injections was reported by Gardner and Pfeiffer (4). Zuckerman (5) produced a prostatic enlargement in rhesus monkeys by the administration of estrone, which was obviated by the addition of testosterone propionate. Rusch (6) obtained a smaller mouse prostate with estrin, which was overcome by testosterone.

On the other hand testosterone and testosterone propionate have been shown to have a cooperative effect with estrogenic substances in restoring atrophic sexual organs to or toward the normal weights in adult ovariectomized rats (7, 8, 9). Thymus involution in mature ovariectomized female rats was greatest when both male and female sex hormones were given (8, 9).

In previous papers the effects of theelin on body growth and organ weights of the female albino rat have been studied (10, 11). The problem came to mind of determining whether testosterone, combined with theelin, would alter the changes produced by theelin in the immature normal female rat. In the present investigation the effects of testosterone propionate and theelin, administered separately and combined, upon the body weight and the weights of the endocrine glands and uterus are reported. As in the previous studies, statistical methods were utilized in interpreting the results.

MATERIALS AND METHODS

Immature female Wistar albino rats were divided into 4 groups of 15 animals each. The hormone dosages at each injection for the various test groups were: 400

I.U. (120 R.U.) theelin in oil,[1] 1 mg. of testosterone propionate (oreton), and a combination of the two hormones. The controls received 0.2 cc. of olive oil. All medication was administered subcutaneously every other day. The first injections were made on the 21st day of age and the last ones on the 29th day of age. The animals were killed and autopsied on the 31st day of age.

It should be mentioned that testosterone propionate is more potent than testosterone and is the most potent androgenic compound known.

The animals were observed and weighed daily, and the age of vaginal patency noted. At the time of autopsy the weights of the hypophysis, thyroid, thymus, ovaries, adrenals and uterus were determined, as well as the body weight, head weight and tail and body lengths. The tissues were placed in Bouin's fixative. The ovaries and uterine horns were imbedded in paraffin, sectioned 10 micra thick, and stained with hematoxylin and eosin for study.

The biometrical interpretation employed was that of Fisher's short-sample method. A value of P of 0.05 or less is regarded as being mathematically significant.

RESULTS

Mean values and other statistical data are recorded in tables 1 and 2.

Uterus. Compared with the controls, the uterus of the theelin group was 164.8% heavier; that of the testosterone propionate group, 309.6% heavier and that of the both-hormone group, 479.7% heavier (fig. 1–4). All changes were mathematically significant.

TABLE 1. MEAN VALUES

Organ	Olive oil	Theelin	Testosterone	Both hormones
Uterus, wt., gm.	0.0491 ± 0.0048	0.1301 ± 0.0051	0.2013 ± 0.0059	0.2849 ± 0.0179
Thymus, wt., gm.	0.4879 ± 0.0213	0.3662 ± 0.0121	0.3216 ± 0.0129	0.2333 ± 0.0140
Hypophysis, wt., gm.	0.0033 ± 0.0001	0.0042 ± 0.0002	0.0034 ± 0.0001	0.0033 ± 0.0001
Thyroid, wt., gm.	0.0066 ± 0.0004	0.0071 ± 0.0003	0.0070 ± 0.0004	0.0077 ± 0.0004
Suprarenals, wt., gm.	0.0164 ± 0.0006	0.0144 ± 0.0004	0.0160 ± 0.0005	0.0164 ± 0.0004
Ovaries, wt., gm.	0.0191 ± 0.0005	0.0195 ± 0.0010	0.0192 ± 0.0009	0.0199 ± 0.0007
Body wt., gm.	68.2 ± 1.9	65.5 ± 1.7	74.7 ± 2.2	68.9 ± 1.6
Head wt., gm.	8.5 ± 0.2	8.4 ± 0.1	8.9 ± 0.2	8.6 ± 0.1
Body length, cm.	13.8 ± 0.1	13.7 ± 0.1	14.2 ± 0.1	13.9 ± 0.1
Tail length, cm.	11.5 ± 0.2	11.5 ± 0.1	11.7 ± 0.2	11.1 ± 0.1

TABLE 2. STATISTICAL SUMMARY (Other groups compared with controls)

Organ	Theelin		Testosterone		Both Hormones	
	% Change	P	% Change	P	% Change	P
Uterus	+164.8	0.01	+309.6	0.01	+479.8	0.01
Thymus	−24.9	0.01	−34.1	0.01	−52.2	0.01
Hypophysis	+26.1	0.01	+2.6	0.66	−0.2	0.9
Thyroid	+6.6	0.43	+5.8	0.54	+15.3	0.1
Suprarenals	−12.3	0.01	−2.3	0.60	−0.1	0.9
Ovaries	+1.8	0.77	+0.6	0.90	+3.9	0.42
Body weight	−3.9	0.32	+9.5	0.04	+1.1	0.77
Head weight	−1.4	0.65	+4.7	0.18	+0.8	0.79
Body length	−0.8	0.56	+2.5	0.10	+0.5	0.69
Tail length	−0.4	0.85	+1.9	0.36	−4.1	0.05

[1] We wish to express our gratitude to Dr. E. Schwenk of Schering Corporation for the supply of testosterone propionate; and to Dr. O. Kamm of Parke, Davis & Company for the supply of theelin.

Contrasted with the controls, the uteri of the theelin-treated rats showed greater diameters, thicker walls (due to increase in muscularis and endometrium), larger lumina, larger glands, and taller epithelium (fig. 5, 6).

Compared with the theelin group, the uterine horns of the testosterone propionate-treated animals displayed greater diameters, thicker muscularis, larger and more irregular lumina, and more vascular though less cellular endometria, with more tortuous glands (fig. 6, 7). The differences were qualitative as well as quantitative.

The administration of both hormones resulted in uterine horns with the greatest diameters and lumina. The constituents of the walls appeared com-

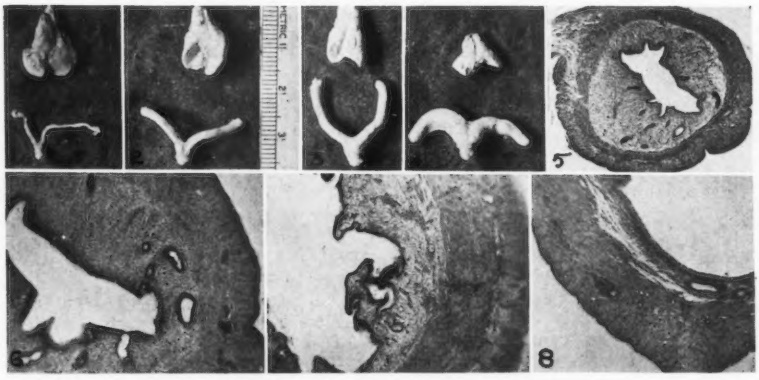


Fig. 1. Uterus and thymus of control rat. Fig. 2. Uterus and thymus of theelin-treated rat. Fig. 3. Uterus and thymus of testosterone propionate-treated rat. Fig. 4. Uterus and thymus of both-hormone-treated rat. Fig. 5. Section through uterus of control rat. ×48. Fig. 6. Section through uterus of theelin-treated rat. ×48. Fig. 7. Section through uterus of testosterone propionate-treated rat. ×48. Fig. 8. Section through uterus of both-hormone-treated rat. ×48.

pressed by the intra-luminal fluid. Areas of vacuolated cells in the epithelium were visible (fig. 8).

Uterine enlargement as early as 72 hours after testosterone propionate injection in immature normal rats was reported by Salmon (12). He noted marked hypertrophy of musculature and proliferation of the endometrium. The changes were less marked in ovariectomized rats. Uterine enlargement in immature rats following administration of androgenic compounds has also been observed (13). Similar results using immature albino mice have been obtained (14).

Uterine enlargement has been obtained as a result of testosterone or testosterone propionate therapy in adult rats (2, 7, 15, 16). An increase in the myometrium and mucosa was noted.

An enlargement of the uterus following androgenic administration in ovariectomized rats has been reported (7, 8, 9, 13, 16).

In mature ovariectomized rats a synergistic effect upon uterine enlargement was discovered when testosterone or testosterone propionate and estrone were administered simultaneously (7, 8, 9). In the present investiga-

tion, the size of the uterus of immature rats showed manifold increase as a result of the combined action of theelin and testosterone propionate.

Following prolonged testosterone propionate therapy, Mazer and Mazer (17) found smaller uterine horns.

Thymus. A comparison with the controls showed the thymus of the theelin injected group to be 24.9% smaller; that of the testosterone propionate, 34.1% smaller and that of the animals receiving both hormones, 52.2% smaller (fig. 1–4). All changes were statistically significant. It is to be noted that testosterone propionate was more effective than theelin, and the combination produced the greatest change.

An increased rate of involution of the thymus by testosterone and testosterone propionate has been shown in adult rats (2, 8, 9, 15). The same change was found in immature rats following prolonged injection (17).

The rate of involution was enhanced by the addition of estrone to testosterone-treated adult ovariectomized rats (8, 9). Our findings corroborate this thymic reaction in the case of normal immature female rats. The thymus was reduced 52% in size within 10 days time. Theelin has been shown to increase the rate of involution of the thymus (10, 11).

Hypophysis. The hypophysis of the theelin group was increased in weight by 26% when compared with the controls. The change was biometrically significant. The administration of testosterone propionate, separately or combined with theelin, had no effect upon pituitary weight, or in other words, prevented the enlargement produced by theelin.

A number of investigators have previously shown that injection of theelin for short periods of time produced an enlargement of the hypophysis (10).

Wolfe and Hamilton (3), working with immature female rats, found no change in pituitary weight following testosterone propionate administration. They reported that the enlargement following estrone therapy was negated by testosterone propionate in mature animals. In mature female rats given a combination of testosterone propionate and estradiol benzoate for a period of 10 days (18), the hypophysis was smaller in weight than that of animals which received estrogenic hormone alone. Mazer and Mazer (17) found a smaller pituitary weight following prolonged testosterone propionate injection in immature rats. The same investigators found no change in adult rats. McEuen, Selye and Collip (19) procured no hypohyseal hypertrophy following testosterone therapy in young rats. Other investigators (8) produced a smaller hypophysis in adult ovariectomized rats with testosterone. The addition of estrone prevented the change. Korenchevsky et al. (9) found no change in hypophysis weight of ovariectomized rats following testosterone administration.

Suprarenals. Theelin injections produced a 12.3% reduction in the size of the adrenals. The difference was statistically significant. In a previous investigation the authors were unable to find a significant change in adrenal weight, though the same dosage was employed (10).

Testosterone propionate, administered alone and in combination with

theelin, produced no significant adrenal weight change. Thus, the addition of the male hormone to theelin prevented the changes evoked by the female hormone.

In ovariectomized adult female rats a lower adrenal weight with testosterone injection, which was prevented by estrone, was reported (8). A decrease in adrenal weight of ovariectomized rats with testosterone propionate, which estrone did not prevent has been produced (9). Smaller adrenals in adult female rats treated daily with 500 gamma of testosterone propionate have been found (2). In immature and adult female rats, prolonged administration of testosterone propionate resulted in a smaller adrenal (17).

Thyroid. The thyroid glands of the several test groups were larger, though not statistically significant, than those of the controls. The increase in the both-hormone group was greatest, being 15% greater than the control series.

In one investigation the thyroid weight was increased in ovariectomized rats following testosterone therapy (8), while in another report no thyroid changes were found (9).

Other organs. In none of the groups were there any noteworthy alterations in the weights of the ovaries or head. Body length likewise remained unchanged.

In adult rats more and/or enlarged corpora lutea have been found following testosterone or testosterone propionate therapy (2, 16, 18). Follicular growth and corpora lutea in immature rats following testosterone propionate treatment were noted (12). Similarly, follicular enlargement was produced in mice, but no corpora lutea (14). Smaller ovaries resulted from longer periods of injection or larger dosages (17, 19).

In the present investigation no corpora lutea were found in the ovaries of the 31-day-old females. There appeared to be a slight gonadotropic effect upon the rat ovary of the testosterone-treated and both-hormone groups, since the largest follicles were discovered there.

Body weight. The body weight was 9.48% greater in the testosterone-treated rats than in the controls—a significant increase. It should be mentioned that the animals of the testosterone propionate group were slightly smaller in initial body weight. The administration of theelin alone resulted in a 3.9% smaller weight, though not mathematically significant. Theelin combined with testosterone propionate had no appreciable influence upon body weight, save to nullify changes produced by testosterone propionate. In a previous investigation (10) the authors produced a significantly lower body weight with theelin, using the same dosage.

A number of investigators found no appreciable alteration in rat body weight as a result of testosterone and testosterone propionate therapy (2, 8, 15, 17, 19). It was reported that 17-methyl testosterone gave a significant increase in guinea-pig body weight (20). Fat deposition was decreased by testosterone propionate (2, 9). A decrease in gain of body weight in ovariectomized rats was reported (9).

Tail length. There was no change in tail length in any of the groups except

the one which received a combination of theelin and testosterone propionate, where the tail was 4.1% shorter. This difference was biometrically significant.

Vaginal patency. The vaginas of the theelin-treated group opened at an average age of 24.5 days, or approximately 84 hours after the first injection. The testosterone propionate-treated rats had open vaginas at a mean age of 25 days, or 96 hours after the first injection. When theelin and testosterone propionate were administered simultaneously, the vaginas became patent at an average age of 24.2 days, or about 77 hours after the initial injection. In no cases were the vaginas of the controls patent.

It is apparent from the data that testosterone propionate was not as effective as theelin in the doses employed. However, the combination of theelin and testosterone propionate displayed a slight synergistic action by having opened the vaginas a few hours earlier than did either hormone alone.

Androgenic compounds have been shown to evoke a premature opening of the rat vagina (12, 13, 21).

DISCUSSION

It has been shown by a number of investigators, cited above, that testosterone propionate and testosterone to a lesser degree, produced an enlargement of the uterus when injected for a relatively short period of time into adult (normal and ovariectomized) rats. In our findings testosterone propionate produced a larger uterus in the immature rat than did theelin. The effect was qualitative (fig. 6, 7), as well as quantitative.

It has been shown by others that the uterine changes occurred in the absence of the ovaries, as well as in the intact animal. Also, the gonadotropic potency of the pituitary was reduced by testosterone and testosterone propionate as early as 10 days after the onset of therapy (22, 23, 24). The reduction was roughly proportional to the dosage. Furthermore, in the present analysis there was only slight evidence of ovarian stimulation, though the uterus displayed definite and marked signs of stimulation. From these statements, it seems justfiable to suggest that most of the action of testosterone propionate was directly upon the female genital tract, rather than indirectly through the pituitary and ovary of the immature rat.

Other experiments have demonstrated a synergistic action between estrone and testosterone upon the genital tract of adult ovariectomized rats. In the present investigation the synergism was evoked upon the reproductive tract of immature intact female rats. It may well be that the apparent cooperative effect between the two hormones was merely independent action in one another's presence. Certainly, there was no antagonistic action on the genital tract.

The thymus displayed marked susceptibility to theelin and testosterone propionate. A 52% reduction in its size in 10 days bears witness of the synergy, or at least lack of opposition, between theelin and testosterone propionate.

None of the investigators noted any significant alteration in body weight

of the rat as a result of androgenic administration, save a check in weight gain and fat deposition in ovariectomized rats. The present data point toward an antagonistic action between the male and female sex hormone upon body weight. Theelin inhibited body weight gain (10, 11). Testosterone propionate enhanced body weight gain in the present work, while a combination of both hormones resulted in a body weight gain similar to that of the controls. It may be that testosterone propionate favored the release of increased amounts of the growth-promoting hormone from the hypophysis, while theelin had no such action.

Hypophyseal enlargement, produced by theelin, was prevented by the addition of testosterone propionate. The decrease in adrenal size, resulting from theelin, was obviated by the addition of testosterone propionate.

The data obtained from the present work demonstrated an inverse relationship as to size between the thymus and the uterus. In the both-hormone group of animals, possessing the largest uterus, the thymus presented the greatest involution. On the other hand, the control animals had the smallest uterus along with the largest thymus. The other groups showed intermediate changes between the two extremes.

That larger doses of theelin might have the same action upon the uterus and thymus is a possibility. However, a comparison of figures 6 and 7 shows that the actions of theelin and testosterone propionate upon the uterus differed qualitatively, as well as quantitatively.

SUMMARY AND CONCLUSIONS

Sixty immature female Wistar albino rats were divided into 4 groups of 15 animals each. The groups, injected subcutaneously every other day, received the following doses at each injection: 400 I.U. (120 R.U.) of theelin in oil, 1 mg. of testosterone propionate (oreton), and the same amounts of the two hormones combined. The controls received 0.2 cc. of olive oil. The animals received the first injection on the 21st day of age, and the last, on the 29th day of age. They were killed on the 31st day of life.

The uterus was increased in size while the thymus was decreased by the treatment. Theelin and testosterone propionate combined presented the greatest change. Testosterone propionate produced a greater change than theelin.

The pituitary weight was increased while the adrenal weight was decreased by theelin, but not by testosterone propionate or the two hormones together.

No significant changes were produced in the weights of the ovaries and head or in the body length.

The thyroid weight was increased in all of the test groups though not significantly. The both-hormone group had the largest glands.

Body weight gain was significantly enhanced by testosterone propionate. Theelin decreased body weight gain though not significantly so in this investigation. The combination of the hormones made the body weight gain parallel that of the control series.

Histologically the ovaries of the testosterone propionate and both-

hormone groups contained the largest follicles. No corpora lutea were found in any instance.

The vaginas were prematurely opened by the hormones.

Antagonistic action between the two hormones was evidenced by changes produced in the body weight, hypophysis and adrenals.

REFERENCES

1. Robson, J. M.: *Proc. Soc. Exper. Biol. & Med.* 35: 49. 1936.
2. Korenchevsky, V., M. Dennison and K. Hall: *Biochem. J.* 31: 780. 1937.
3. Wolfe, J. M., and J. B. Hamilton: *Endocrinology* 21: 603. 1937.
4. Gardner, W. U., and C. A. Pfeiffer: *Proc. Soc. Exper. Biol. & Med.* 38: 599. 1938.
5. Zuckerman, S.: *Lancet* 231: 1259. 1936.
6. Rusch, H. P.: *Endocrinology* 21: 511. 1937.
7. Korenchevsky, V., and K. Hall: *J. Path. & Bact.* 45: 681. 1937.
8. Korenchevsky, V., M. Dennison and I. Brovsin: *Biochem. J.* 30: 558. 1936.
9. Korenchevsky, V., M. Dennison and M. Eldridge: *Biochem. J.* 31: 475. 1937.
10. Freudenberger, C. B., and F. W. Clausen: *Anat. Rec.* 69: 171. 1937.
11. Freudenberger, C. B., and F. W. Clausen: *Anat. Rec.* 68: 133. 1937.
12. Salmon, U. J.: *Endocrinology* 23: 779. 1938.
13. Deanesly, R., and A. S. Parkes: *British M. J.* 1: 257. 1936.
14. Starkey, W. F., and J. H. Leathem: *Proc. Soc. Exper. Biol. & Med.* 39: 218. 1938.
15. Korenchevsky, V., M. Dennison and K. Hall: *Biochem. J.* 31: 1434. 1937.
16. Nelson, W. O., and C. G. Merckel: *Proc. Soc. Exper. Biol. & Med.* 36: 823. 1937.
17. Mazer, M., and C. Mazer: *Endocrinology* 24: 175. 1939.
18. Wolfe, J. M., and J. B. Hamilton: *Proc. Soc. Exper. Biol. & Med.* 37: 189. 1937.
19. McEuen, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.* 36: 390. 1937.
20. Bottomley, A. C., and S. J. Folley: *J. Physiol.* 94: 26. 1938.
21. Butenandt, A., and H. Kudzus: *Ztschr. f. Physiol. Chem.* 237: 75. 1935. (Cited by Salmon, U. J.: *Proc. Soc. Exper. Biol. & Med.* 37: 488. 1937.)
22. Hertz, R., and R. K. Meyer: *Endocrinology* 21: 756. 1937.
23. Cutuly, E., and E. C. Cutuly: *Endocrinology* 22: 568. 1938.
24. Hamilton, J. B., and J. M. Wolfe: *Endocrinology* 22: 360. 1938.

VASCULAR REACTIONS OF THE UTERUS OF THE IMMATURE RAT[1]

R. B. HOLDEN

From the Biological Laboratories, Harvard University

CAMBRIDGE, MASSACHUSETTS

MANY WORKERS have reported uterine hyperemia or 'increased vascularity' following the administration of estrogen. There has been no clear agreement, however, on the time-relations of the hyperemia and no work has appeared on the details of this effect in the rat. The rate of development of this hyperemia in immature rats, its duration and regression have been studied with a view to ascertaining, if possible, the relation of the hyperemia to the other effects of estrogen. Drugs of varied vasomotor action were tested in an exploratory investigation of the possible mechanism of the reaction.

METHODS

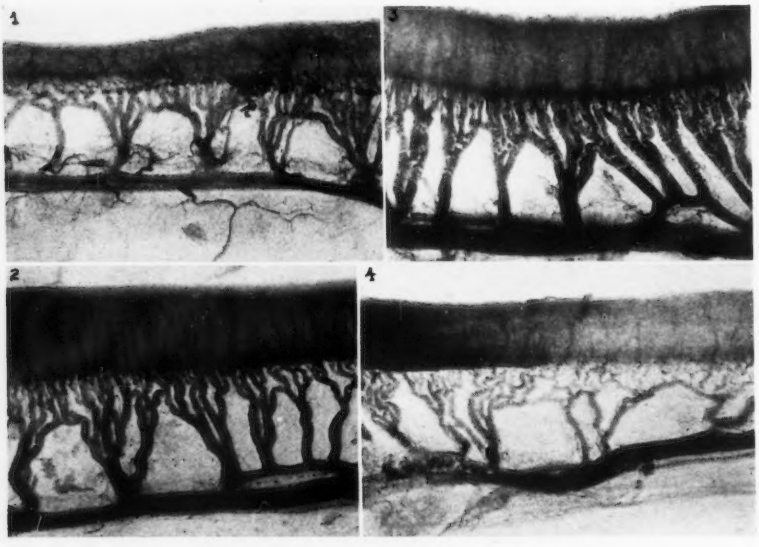
Albino rats, 21 to 23 days old, were used throughout. Their uterine vessels were studied by two methods, the intravenous injection of a non-toxic, opaque substance and by direct observation of the transilluminated uterus. A series of rats was given a single injection of 0.1γ estradiol subcutaneously and at intervals thereafter were injected, under ether, through a femoral vein with 1 cc. of an 8% colloidal suspension of black mercuric sulfide (1). Bouin's fixative was injected shortly afterward into the peritoneal cavity and the uterus and mesometrium were subsequently dissected. On clearing in benzyl benzoate these uteri show the lumina of the blood vessels clearly outlined by the mercuric sulfide.

Supplementing this, the vascular reactions of living vessels were studied by direct microscopic observation. A narrow Lucite rod, curved gently at its tapered end, transmitted cold light from a small 8-volt D.C. lamp. The tip was placed directly beneath the uterus which was exposed through a small lumbo-dorsal incision. With the rat under nembutal anesthesia and with the exposed parts kept moistened with Locke's solution the preparation could be maintained for 4 to 6 hours. Thus the mesometrial and uterine vessels, their changes in size and the rate of flow through them could be easily observed under a dissecting microscope. Drugs of varied vasomotor action, in different dilutions, were applied directly to the uterus and the reactions of the vessels to them were noted. Rats given a single subcutaneous injection of estradiol could be observed at intervals afterward in this manner. Thus results from the two methods could be compared.

[1] Aided by a grant from the Rockefeller Foundation administered by Professor F. L. Hisaw.

RESULTS

Estrin-induced hyperemia could be seen to begin with vasodilatation at about the second hour. By the third hour it had unquestionably begun (fig. 1, 2). Direct observations up to the third hour, owing to errors of subjective observation, did not show concisely and at once the difference between the normal and the estrin-treated uterus. The vasodilatation increased progressively until the sixth hour when it was maximal (fig. 3). Direct observation at the sixth hour revealed a red, edematous uterus in whose dilated vessels blood was flowing rapidly and profusely. After the sixth hour the effect gradually regressed until by the 24th hour it had largely disappeared (fig. 4).



CLEARED WHOLE MOUNTS OF THE UTERI OF IMMATURE RATS INTRAVENOUSLY INJECTED IN VIVO WITH BLACK MERCURIC SULFIDE. The large vessels which parallel the uterus are the uterine artery and vein lying in the mesometrium. Above them is the uterine plexus. Fig. 1. Uterus of a normal untreated rat. Note the slender uterus. Fig. 2. Uterus of a rat 3 hours after a single injection of 0.1γ estradiol. Fig. 3. Uterus of a rat 6 hours after a single injection of 0.1γ estradiol. Maximal vasodilatation. The living uterus is markedly hyperemic. Fig. 4. Uterus of a rat 24 hours after a single injection of 0.1γ estradiol. The effect has largely disappeared. Note the slender uterus.

The concurrent administration of atropine did not inhibit this vasodilatation. After 7½ hours the uteri of rats given 0.1γ estradiol showed no significant difference from those given 1 mg. of atropine in addition to the estradiol. Even the enormous dose of 8 mg. of atropine did not inhibit the effect. Acetyl choline in dilutions greater than 1:200 usually had no effect when applied topically to the uterus, although solutions of 1:1000, 1:10,000 and 1:1,000,000 were tested. Strong solutions (1%) of this substance, contrary to expectation, produced a marked but brief vasoconstriction lasting

about 1½ minutes. Atropinization of the uterus before the local application of a 1% solution of acetyl choline prevented the blanching which otherwise would have been produced. Atropine itself had no observable vasomotor effect on the vessels of the normal uterus nor on those exhibiting any stage of the estrin-hyperemia. Eserine, in dilutions of 1:1000 and 1:100,000 had no noticeable effect of itself nor did it affect the action of acetyl choline solutions. Likewise, yohimbine, reputedly a specific vasodilator of the genital tract, had no effect in a concentration of 1:100 applied directly nor when 1 mg. was given subcutaneously.

Ethoxycholine and mecholyl, choline derivatives, produced a vasoconstriction lasting about 1½ minutes when locally applied in a dilution of 1:100. Solutions of the dilution 1:10,000 had no effect. Like acetyl choline these drugs were inhibited by atropine.

Other vasoconstrictors of uterine vessels are pituitrin and epinephrine. Pituitrin, applied locally, caused a blanching which lasted about 2½ to 3 minutes. Epinephrine, in dilutions of 1:1000 to 1:10,000,000, produced a marked vasoconstriction which lasted 9½ to 10 minutes. This was true of both normal and estrin treated uteri.

The blanching of the uterus produced by vasoconstrictors could be rather abruptly terminated by the local application of histamine in dilutions of 1:100 or 1:1000. Histamine rendered the normal uterus red and hyperemic. Except for padutin, of whose properties only cursory tests have been made, histamine is the only substance yet tried which produced, when locally applied, a vasodilatation of uterine vessels.

DISCUSSION

It is well known that estrin produces an increase in the weight of the uterus of the immature rat and that there is a concomitant hyperemia. It was shown by Astwood (2) that the early increase in weight and water content of the uterus following a single injection of estradiol begins about the second hour and rises to a maximum at the sixth. Following this, the percentage of water regresses with minor fluctuations until, by the 42nd hour, it is again normal. This study shows that the vasodilatation following a single injection of 0.1γ estradiol closely parallels the early increase in water content of the uterus. Direct observation of the uterus of estrin-treated rats for one hour immediately following the injection failed to reveal any immediate vasodilator effect like that found in rabbits by Markee (3). Reynolds (4) finds that in the rabbit following estrogen there is an increase in acetyl choline content which coincides with the initial vasodilatation of Markee, and suggested that in the rabbit acetyl choline may mediate the estrogen effect. The finding that acetyl choline produces vasoconstriction and the fact that atropine fails to inhibit the estrin-hyperemia whether seen in injected or living vessels indicates that acetyl choline is not a mediator of the effect in the rat.

It is difficult to see, since the vasodilatation so closely parallels the increase in water content, whether the primary effect of the estradiol is

vascular or is on the specific uterine cells. Since estradiol has no known direct vasomotor action, it is logical to assume at first that the hyperemia results from the liberation of some vasodilator substance from specific uterine cells. This is probably not acetyl choline. It is possibly a histamine-like substance, for the only biological substance found to produce dilation of these vessels was histamine. Histamine has been found in many organs of the animal body (5) and the limitation of the hyperemia to the genital tract suggests some locally produced vasodilator whose range is limited by the activity of a destructive agent, perhaps histaminase (6).

SUMMARY

The vascular responses of the uterus of the immature rat have been studied by the intravenous injection of mercuric sulfide in vivo and by direct microscopic observation of living vessels transilluminated by means of a Lucite rod. Following the single injection of 0.1γ estradiol there results a uterine hyperemia, the development of which is shown to parallel closely the early increase in weight and water content of the uterus which are likewise induced by estrin. Various drugs were tested for their vasomotor effects. From these tests it was evident that these estrin effects on the vessels were not mediated by acetyl choline. Evidence is given that the mediator may be an H-substance. Pituitrin, epinephrine and choline derivatives cause vasoconstriction. Eserine, atropine and yohimbine had no vasomotor effect. Padutin and histamine produce vasodilatation.

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REFERENCES

1. Potter, T. S.: *Anat. Rec.* 58: 197. 1931.
2. Astwood, E. B.: *Endocrinology* 23: 25. 1938.
3. Markee, J. E.: *Am. J. Physiol.* 100: 32. 1932.
4. Reynolds, S. R. M.: *J. Physiol.* 95: 258. 1939.
5. Best C. H., and E. W. McHenry: *Physiol. Rev.* 11: 371. 1931.
6. Best, C. H., and E. W. McHenry: *J. Physiol.* 70: 373. 1930.

DIETHYLSTILBESTROL COMPARED TO ESTRONE IN CAUSING ESTRUS IN SPAYED MICE; AND IN CONJUNCTION WITH PROGESTIN IN INDUCING SEXUAL RECEPTIVITY IN SPAYED GUINEA PIGS

J. A. LEIGHTY AND H. J. WICK
From the Lilly Research Laboratories
INDIANAPOLIS, INDIANA

In 1938, Dodds, Golberg, Lawson and Robinson (1) reported the synthesis of 4:4′-dihydroxy-α, β-diethylstilbene (diethylstilbestrol) and showed it to be 2 to 3 times as potent as estrone in spayed rats. Since then numerous investigators have studied the action of this synthetic substance compared to estrone and various other estrogens. Thus it has been reported to be similar to estrone in its ability to abolish castration signs in the anterior pituitary (2), in producing mammary adenocarcinomas in male mice (3), in bringing about lipemia and calcemia in the cock (4), in inhibiting the comb-growth action of male hormones when given by injection but not when rubbed on the comb (5), in inhibiting lactation in the rat and crop-gland response in the pigeon (6), and in sensitizing the infantile rabbit uterus and monkey uterus to progestin (7, 8, 9).

In 1938, Boling, Young and Dempsey (10, 11) showed that a condition of sexual receptivity in the spayed guinea pig could be induced by the injection of estrone alone, but that a better and more characteristic response was obtained when the estrone injection was followed several hours later by an injection of progestin. It seemed of interest to determine if this new synthetic substance, diethylstilbestrol, would substitute for the natural estrogen in producing this response in the spayed guinea pig when injected like estrone, and also when applied percutaneously.

In order to be certain of the estrogenic activity of the diethylstilbestrol used, we have made a comparison of this substance to estrone in the spayed mouse. Both substances have been administered in 3 different ways, namely, subcutaneously, orally and percutaneously.

EXPERIMENTAL

The spayed mice used in this work had been on routine test with estrone for several months. All comparisons were made with pure crystalline estrone. The following procedures were used with the mice.

A. One group of animals was injected with varying amounts of estrone dissolved in water. The injections were made 3 times in one day and vaginal smears were taken

on the third day. A mouse was considered to be positive (+) (in estrus) if at least 50% of the smear cells were cornified and no leucocytes were present. If this condition were not fulfilled, then the animal was given a negative (−) rating. A mouse unit of estrone under these conditions is the amount which will bring 50% of a group of spayed mice into estrus. Diethylstilbestrol was tested in the same way. The total volume of solution per mouse was 1.0 cc.

B. One group of animals was treated orally with estrone in water 3 times in one

TABLE 1. ACTION OF DIETHYLSTILBESTROL COMPARED TO ESTRONE IN THE SPAYED MOUSE

Estrone				Diethylstilbestrol			
Total dose per mouse γ	Method of administration	Number of animals +	Number of animals −	Total dose per mouse γ	Method of administration	Number of animals +	Number of animals −
0.05	Subcut.	10	10	0.025	Subcut.	0	20
0.05	Subcut.	11	9	0.05	Subcut.	1	19
0.25	Orally	0	5	0.058	Subcut.	3	17
0.50	Orally	0	6	0.066	Subcut.	12	8
1.00	Orally	3	17	0.05	Orally	0	5
1.00	Orally	6	14	0.25	Orally	2	4
1.25	Orally	9	10	0.30	Orally	11	9
1.25	Orally	4	15	0.375	Orally	6	0
0.05	Percut.	0	5	0.05	Percut.	0	5
0.075	Percut.	1	4	0.15	Percut.	0	5
0.10	Percut.	9	9	0.30	Percut.	3	4
0.15	Percut.	5	0	0.35	Percut.	15	5

TABLE 2. EFFECT OF PERCUTANEOUS ADMINISTRATION OF ESTROGEN FOLLOWED BY A SINGLE INJECTION OF PROGESTIN IN CAUSING HEAT IN SPAYED GUINEA PIGS

Total amt. of estrogen rubbed on	Amt. of progestin injected	Number treated	Number receptive	Mean latent period	Mean length of receptive period	Combined latent and receptive periods
γ	I.U.			*hr.*	*hr.*	*hr.*
Estrone						
10	0.1	10	9	5	7.2	12.2
10	0.05	10	8	4.7	7.4	12.1
10	0.03	10	9	4.4	10.3	14.7
Diethyl-stilbestrol						
10	0.1	10	10	5.4	8.7	14.1
10	0.05	10	7	5.4	8.8	14.2
10	0.03	10	8	5.0	8.0	13.0

day, and vaginal smears were taken on the third day. The total volume of solution fed per mouse was 1.0 cc. Diethylstilbestrol was tested in a similar manner.

C. One group of animals was treated with estrone in cottonseed oil by the percutaneous method. The oil was rubbed on the shaved necks of the animals 3 times in one day. The total dose was 0.03 cc. per mouse. Smears were taken on the third day. Diethylstilbestrol was administered in the same way. The results of these experiments are shown in table 1.

The spayed guinea pigs used in this work had all been treated routinely every 2 weeks for several months with estrone followed by progestin in order to determine their responsiveness.

It was found early in this work that repeated injections of these oil solutions

caused hard lumps under the skin of the animals and since Zondek (12) had shown estrone to be readily absorbed through the skin of mice, we decided to try this percutaneous method of administration on the guinea pigs. In this method of administration the estrone and the diethylstilbestrol were dissolved in oil and rubbed on the shaved necks of the animals. The oil solutions were rubbed on 4 times during 2 days and the progestin in oil was injected on the morning of the third day. The total amount of oil solution rubbed on each animal was 0.1 cc. The results are summarized in table 2. In order to be sure that our animals were responding similarly to those of

TABLE 3. EFFECT OF A SINGLE INJECTION OF ESTROGEN FOLLOWED BY A SINGLE INJECTION OF PROGESTIN IN CAUSING HEAT IN SPAYED GUINEA PIGS

Amount of estrogen administered	Amount of progestin administered	Time between injections	Number injected	Number receptive	Mean latent period	Mean length of receptive period
γ	I.U.	*hr.*			*hr.*	*hr.*
Estrone						
20	0.2	24	3	3	5.5	6.0
5	0.2	24	3	3	6.3	4.5
5	0.1	48	5	1	5.0	4.0[1]
5	0.05	48	5	1	5.0	4.0[1]
Diethyl-stilbestrol						
20	0.2	24	3	3	5.8	5.0[1]
5	0.2	24	3	3	6.2	4.8[1]
5	0.1	48	5	3	5.5	6.8
5	0.05	48	5	3	5.5	5.6

[1] Animals still receptive.

Dempsey and his coworkers (10, 11), we injected a few animals with estrone and diethylstilbestrol dissolved in oil. These results are summarized in table 3.

RESULTS AND DISCUSSION

Subcutaneous and percutaneous methods of administration in the mice gave consistent results. Inspection of table 1 shows that 0.05 γ of estrone is one mouse unit when given subcutaneously under the conditions of our experiment. It required 25 to 30 times as much orally and 2 times as much percutaneously to get the same effect. When stilbestrol was tested in the same way it required 1.25 times as much subcutaneously, 6 times as much orally and 6 to 7 times as much percutaneously to get an estrogenic effect equivalent to that of 0.05 γ of estrone subcutaneously. These results are not in complete agreement with those of Emmens (13), who reported that under the conditions of his experiment 0.1 γ of either substance was the amount necessary to cause estrus in 50% of his animals. Furthermore, he found that orally it required 10 times as much estrone and 4 times as much stilbestrol to get an estrogenic effect equivalent to that obtained when these substances were given subcutaneously. We have observed many times that it requires 0.1 γ of estrone as a mouse unit when the mice are injected for the first time following ovariectomy; however, on subsequent routine use the mice respond to 0.05 γ of estrone.

In table 2 one can observe that the results obtained with the spayed guinea pigs using the percutaneous administration of these two substances followed by the subcutaneous administration of progestin in oil are similar. In all this work the latent period and the receptive period are those defined by Dempsey and coworkers (10, 11). The latent period is the time between the injection of the progestin and the first definite response of receptivity; and the receptive period is the time the animal will respond when stroked in the dorso-lumbar region. The results obtained by injecting diethylstilbestrol followed by progestin (table 3) compare favorably with those obtained with estrone followed by progestin. The results obtained with estrone and progestin are similar to those reported by Dempsey and coworkers (10, 11), although not as extensive. Progestin in oil has also been rubbed on a few animals instead of being given by injection, however, the data thus far are insufficient to report other than the fact that we have used large doses to obtain good results. This work is being continued.

SUMMARY

Diethylstilbestrol, a new synthetic estrogen, has been compared to pure estrone in the spayed mouse and spayed guinea pig. It was found that 0.05 γ of estrone was one mouse unit when given subcutaneously under the conditions of our experiment. It required 25 to 30 times as much orally and 2 times as much percutaneously to get the same effect. When stilbestrol was tested in the same way it required 1.25 times as much subcutaneously, 6 times as much orally and 6 to 7 times as much percutaneously to get an estrogenic effect equivalent to that of 0.05 γ of estrone subcutaneously.

This synthetic substance when followed by progestin was found to induce sexual receptivity in spayed guinea pigs. The results obtained either by injection or percutaneous administration of the estrogen were comparable to those obtained with estrone in the doses investigated.

REFERENCES

1. Dodds, E. C., L. Goldberg, W. Lawson and R. Robinson: *Nature* 141: 247. 1938.
2. Jacobsen, E.: *Endokrinol.* 21: 20. 1938.
3. Lacassagne, A.: *Compt. rend. Soc. de Biol.* 129: 641. 1938.
4. Zondek, B., and L. Marx: *Nature* 143: 378. 1939.
5. Mühlbock, O.: *Nature* 143: 160. 1939.
6. Folley, S., and S. Watson: *Lancet* 2: 423. 1938.
7. Dodds, E. C., W. Lawson and R. L. Noble: *Lancet* 1: 1389. 1938.
8. Palmer, A., and S. Zuckerman: *Lancet* 2: 933. 1939.
9. de Fremery, P., and M. C. Geerling: *Acta Brev. Neerland. Physiol.* 9: 17. 1939.
10. Boling, J. L., W. C. Young and E. W. Dempsey: *Endocrinology* 23: 182. 1938.
11. Collins, W. J., J. L. Boling, E. W. Dempsey and W. C. Young: *Endocrinology* 23: 188. 1938.
12. Zondek, B.: *Lancet* 2: 1107. 1938.
13. Emmens, C. W.: *J. Physiol.* 94: 22P. 1939.

THE INACTIVATION OF ENDOGENOUS ESTROGEN BY THE LIVER[1]

NATHAN B. TALBOT

From the Biological Laboratories, Harvard University and the Department of Pediatrics, Harvard Medical School

CAMBRIDGE AND BOSTON, MASSACHUSETTS

EVANS AND BURR (1) discovered that ovarian hormone was more efficacious when administered subcutaneously than when injected into the peritoneal cavity. The significance of this finding was clarified when Zondek (2) found that estrogen is rapidly inactivated in the animal body. He showed that liver mash could inactivate estrogen in vitro whereas mash from other organs was ineffective. An enzyme, estrinase, was postulated as the substance responsible for the inactivation. Subsequently Israel, Meranze and Johnston (3) have shown by means of an heart-lung-liver perfusion experiment that estrogen is destroyed when the liver is present, but not when the liver is removed from the system. Recently Heller, Heller and Sevringhaus (4) reported that estradiol and estriol were completely inactivated by rat liver slices in vitro. Rat lung, spleen and uterus caused no decrease in potency, while rat kidney caused only a slight decrease. They infer from their experiments that the effective destroyer of estradiol is enzymatic in nature and that this process proceeds best when the intact liver cell is present.

The foregoing evidence suggests that if the liver is the chief organ concerned with the inactivation of estrogen, impairment of liver function should alter the metabolism of estrogenic hormones in the living animal. One method for impairing liver function was suggested by the work of Gardner and his associates (5). They showed that carbon tetrachloride produces definite necrosis of liver cells in 24 hours and maximum injury at 48 hours. Bennett, Drinker and Warren (6) have determined furthermore that the injurious effects of chlorinated hydrocarbons are manifested solely in the liver. The present investigation is a study of the changes occurring in the genital tract of the immature rat after the administration of a mixture of carbon tetrachloride and alcohol. The results give further support to the theory that the liver inactivates estrogen.

EXPERIMENTAL METHODS

Group A. Fifty-one 21- to 25-day-old female rats were included in Group A. They were litter mates from an inbred colony. Their ration consisted of Pratt's dog biscuits, rolled oats, powdered dextrose and water. On the twenty-first day of life 0.1 cc. of an equal mixture of carbon tetrachloride and 95% ethyl alcohol was administered by gavage. Within 24 to 48 hours following this treatment the animals

[1] The work was supported in part by a grant from The Rockefeller Foundation administered by Professor F. L. Hisaw.

became listless, icteric and partially dehydrated. They were most severely prostrated on the second and third days. If more than 0.1 cc. of the carbon tetrachloride mixture was used, the mortality exceeded 50% by 48 hours, whereas if much less was given the animals showed little or no systemic defects and could not be distinguished grossly from normal animals.

At periods of 1, 2, 3 and 4 days following the administration of the carbon tetrachloride, groups of animals were autopsied. It was observed that all the tissues were icteric. The livers were grossly pale pink and mottled in contrast to those of normal animals which are of an homogeneous red color. No significant variation from the normal liver weight was detected. Microscopic sections of the liver revealed scattered areas of focal necrosis. The uteri were found to be edematous and congested. A few were ballooned with intra-uterine fluid. The ovaries and uteri were removed, freed from adjacent tissues and weighed immediately (table 1).

Group B. Group B was composed of 10 rats which were castrated on the twentieth

TABLE 1. UTERINE AND OVARIAN WEIGHT OF IMMATURE RATS 0, 1, 2, 3 AND 4 DAYS AFTER THE ADMINISTRATION OF A MIXTURE OF CARBON TETRACHLORIDE AND ALCOHOL

	Group A—Intact Rats Days after CCl_4–C_2H_5OH given				
	0	1	2	3	4
No. Animals	11	9	15	11	5
Uterine weight, mg. Standard error[1] Range	20 18–22	24 1.25 19.5–29	37 2.2 22–52	48 4.2 29–75	35 5.35 19–48
Ovarian weight, mg. Range	9.2 7.7–.216	7.6 7.0–8.5	8.3 7.5–10.5	7.9 6.0–9.0	6.6 6.0–8.0
	Group B—Castrated Rats				
No. Animals			10		
Uterine weight, mg. Range			17.3 13.0–20.5		

[1] Standard error of the mean= $\sqrt{\frac{\Sigma d^2}{N(n-1)}}$.

day of life, treated with carbon-tetrachloride-alcohol on the twenty-first day and autopsied on the twenty-third day. At autopsy they appeared to be as listless, icteric and dehydrated as the animals in Group A, but their uteri were neither congested nor edematous. Careful examination showed that no part of the uterus had been removed at the time of castration (table 1).

RESULTS

The data (table 1) show that the uterine weight increased after carbon tetrachloride was administered. At the end of 24 hours there was a small but definite average increase over the control weight. An average maximum weight of 48 mg. was attained on the third day. A curve showing the percentage increase in average uterine weight is presented in figure 1. In contrast with this, animals castrated one day prior to carbon tetrachloride therapy show no increase in uterine weight. The ovaries, on the other hand, do not increase in weight.

DISCUSSION

The uterine weight of normal 21- to 26-day-old albino rats in our colony is approximately 20 mg. and seldom varies more than 2 mg. from the average. Astwood (7) has shown that in such rats a single dose of estrogen will cause a marked increase in the uterine weight and water content. The uterine response 6 hours after the administration of estrogen is sufficiently consistent to serve as an accurate assay method. Lawson and coworkers (8) have also demonstrated that the uterine weight response to divided doses of estrogen

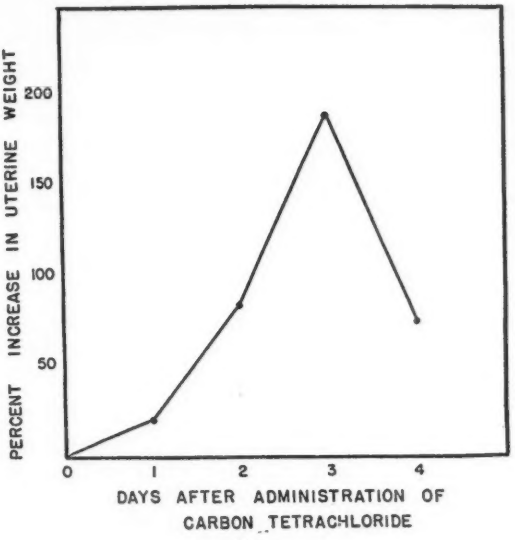

Fig. 1

is consistent. The weight of the uterus in the immature rat may be used, therefore, as an index of the level of estrin in the blood.

The data reported here show that the uteri of intact rats which have been poisoned with carbon tetrachloride (Group A) weigh significantly more than normal. Therefore, it may be inferred that the uteri of the poisoned animals were exposed to an increased amount of estrogen. Because the uteri of castrated animals (Group B) did not increase in weight, it seems probable that the ovaries were the source of the estrogen which caused the uterine weight gain in the intact animals (Group A). These findings suggest, therefore, that the ovaries of immature rats secrete estrogenic hormone, but that in the healthy animal the liver keeps the concentration of circulating estrogen at a low level by inactiving most of that which the ovary produces. The evidence at hand indicates that carbon-tetrachloride-alcohol injures the liver cells to such an extent that they are no longer capable of inactivating even minute amounts of estrogen. The effective level of estrogenic hormone in the blood stream rises as a consequence and causes the uterus to increase in weight.

There is other evidence for the thesis that the ovaries of immature rats secrete estrin. It is generally recognized that the uteri of immature rats tend to atrophy following castration. Recently Kallas (9) and Meyer and Hertz (10) have added other evidence as demonstrated in pituitary-gonadal relationships.

It is admitted that other explanations for the phenomena observed are possible. An inefficient liver might favor pituitary stimulation of the immature ovary. If this were the case, the ovary would produce more estrogen which would in turn cause an increase in uterine weight. The data obtained show, however, that there was no increase in ovarian weight during the periods of observation. There was, if anything, a decrease in the average weight of the ovaries which suggests a suppression of gonadotropic activity.

It is unlikely that carbon tetrachloride itself may be estrogenic. Rats who received one-half to three-quarters of the usual dose of the poison showed neither gross evidence of liver damage nor any increase in the uterine weight. The maximum uterine weight was always observed when the animals appeared to be suffering most from the poison. This fact suggests a correlation between the severity of the poisoning and the uterine response rather than a mutual relationship between the dose of the poison and the weight of the uterus.

SUMMARY AND CONCLUSIONS

The evidence of in-vitro experiments performed by other investigators suggests that the liver is the chief organ responsible for the inactivation of estrogen in the animal body. Intact 21-day-old female rats poisoned with a mixture of carbon tetrachloride and alcohol show signs of acute liver damage within 48 hours. At the time when the animals show the greatest prostration, their uteri are stimulated by an estrogenic substance as evidenced by increased weight, edema and congestion. Castration of the rats prior to the administration of the poison prevents the uterine reaction. From these facts the following conclusions are drawn.

(*a*) The ovaries of 21- to 24-day-old rats normally secrete small amounts of estrogen.

(*b*) In the normal intact animal, the liver inactivates most of the circulating estrogen and thereby keeps the concentration of the substance in the blood stream at a very low level.

(*c*) When the liver is severely poisoned, it is no longer capable of inactivating estrogen efficiently. As a consequence, the concentration of estrogen in the circulation rises and the characteristic uterine response becomes evident.

REFERENCES

1. Evans, H. M., and G. O. Burr: *Am. J. Physiol.* 77: 518. 1926.
2. Zondek, B.: *Skand. Arch. f. Physiol.* 70: 133. 1934.
3. Israel, S. L., D. R. Meranze and C. G. Johnston: *Am. J. M. Sc.* 194: 835. 1937.
4. Heller, C. G., E. J. Heller, and E. L. Sevringhaus: *Proc. Am. Phys. Soc.* April 26, 1939. p. 114.
5. Gardner, G. H., R. C. Grove, E. D. Gustafson, M. J. Thompson, H. S. Wells and P. D. Lamson: *Bull. Johns Hopkins Hosp.* 36: 107. 1925.
6. Bennett, G. A., C. K. Drinker and M. F. Warren: *J. Indust. Hyg. & Toxicol.* 20: 97. 1938.
7. Astwood, E. B.: *Endocrinology* 23: 25. 1938.
8. Lawson, H. D., C. G. Heller, J. B. Golden and E. L Sevringhaus: *Endocrinology* 24: 35. 1939.
9. Kallas, H.: *Klin. Wchnschr.* 9: 1345. 1930.
10. Meyer, R. K., and R. Hertz: *Am. J. Physiol.* 120: 232. 1937.

ON THE ROLE OF THE PITUITARY STALK IN THE REGULATION OF THE ANTERIOR PITUITARY, WITH SPECIAL REFERENCE TO THE THYROTROPIC HORMONE[1]

UNTO U. UOTILA[2]

From the Department of Anatomy, Harvard Medical School

BOSTON, MASSACHUSETTS

NUMEROUS CLINICAL observations seem to indicate that the function of the anterior pituitary is at least partially under the control of the central nervous system. Experimental data, however, on this question are not abundant and the results obtained by different workers vary greatly, apparently as a result of variations in operative methods and in the extent of the lesions made in the anterior pituitary or hypothalamus.

Of the various possible relationships of the pituitary stalk to the anterior pituitary, its influence on the gonadotropic hormones is the best known at present. Some earlier authors (1–5), observed genital dystrophy in dogs and rabbits after section of the pituitary stalk or obstructing it with silver clips. Mahoney and Sheehan (6) also noted genital dystrophy in dogs after interruption of the stalk but failed to find it in monkeys which possess a long stalk. According to Westman and Jacobsohn (7, 8) rabbits and rats develop an ovarian and testicular atrophy similar to that following hypophysectomy but with the notable difference that the ovaries react to prolan with the formation of blood follicles and corpora lutea (18). In contrast to these results, Brooks (9) in his careful studies found that rabbits remain sexually normal after pituitary stalk section except for a failure to ovulate after mating. This latter finding indicates that the discharge of luteinizing hormone may be under hypothalamic control. Dempsey (10) observed that in guinea pigs even ovulation is not disturbed by this procedure.

Thyroid changes after pituitary stalk section have also been studied to some extent. Brooks (9) found no histological changes, whereas Westman and Jacobsohn (11) reported normal histology mixed irregularly with signs of hypoactivity. The basal metabolism is unchanged by stalk section in monkeys (6) and in rats with experimental diabetes insipidus (12).

The adrenal glands are said to be unaffected by cutting the stalk (9, 11).

Mahoney and Sheehan (6) found the blood sugar to be normal in monkeys after this procedure. Brooks (9) confirmed this in the case of rabbits and also noted a normal insulin sensitivity.

The earlier authors reported that with the genital dystrophy and adiposity the animals remained infantile and their growth was delayed. This was also reported in the case of rats (11), but Brooks (9) failed to find any abnormalities in the growth of rabbits.

From this brief review it is apparent that the results of pituitary stalk section, hitherto discovered, are conflicting and in many respects negative. The results suggest, moreover, that any influences of stalk section will be

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[2] Holder of a Finnish Government Fellowship, 1938; Fellow of the Rockefeller Foundation, 1939.

appreciably less in terms of immediate, severe injuries to the various endocrine organs than in less easily demonstrable functional changes which it will be necessary to study by means of sundry functional tests. For example, the findings of Brooks, cited above, indicate that the influences of stalk section on the ovaries are revealed solely by changes in the response of the ovaries to mating.

Consequently, in planning the present experiments, thought was given to the selection of a test applicable to several of the endocrine organs and at the same time conceivably influenced by pituitary stalk section. A suitable functional test for the thyrotropic and adrenocorticotropic factors is available in the reaction to cold.

Selye considers cold as an 'alarming' stimulus which, in acute stages, causes thyroid and adrenal hyperplasia, gonad damage, and inhibition of growth. Thyroid and adrenal hyperplasia are abolished after hypophysectomy (13). Hence the reaction is probably mediated through the anterior pituitary. In a search for possible nervous pathways involved in the thyrotropic and corticotropic cold reaction, the rôle of the cervical sympathetics has been studied (13). It was found that although cervical sympathectomy modified slightly and temporarily the thyrotropic function of the hypophysis and its cold reaction, nevertheless the cervical sympathetics were not necessary for the cold reaction or for normal thyroid function. It was also noted that cervical sympathectomy had no demonstrable effect on the corticotropic function.

The present paper reports the effects of pituitary stalk section combined with the cold reaction. More specifically, the following studies are presented: *a*), the effects of stalk section on the thyroid, adrenals, gonads and growth; *b*), the effect of stalk section followed by cold on these glands and *c*), the effect of cold after blank operations on these glands.

MATERIAL AND METHOD

Seventy-five male albino rats of Wistar strain weighing 120 to 130 gm. were used, 27 as experimental animals, 48 as different controls.

All pituitary stalk sections were done under ether anesthesia, using the parapharyngeal approach for hypophysectomy. An opening in the skull was made with a dental drill a little cranial to the spheno-occipital junction. The dura was then opened and the pituitary stalk and posterior lobe exposed to view. The stalk was severed with a small hook. After the operation a few animals developed a general pituitary insufficiency appearing as an atrophy of the other endocrine organs. This insufficiency was found only after extensive inadvertent damage to the anterior pituitary, as was shown by serial sections of the hypothalamo-hypophyseal region. It did not occur after successful pituitary stalk section, and hence such cases of insufficiency were discarded. Hypothalamic lesions were seldom seen in the serial sections. Most animals developed symptoms of diabetes insipidus for a variable time, but no special tests were done to verify the diagnosis. Many successful pituitary stalk operations were followed by complications such as pneumonia, hemorrhage, etc., which significantly reduced the number of experimental animals. Blank control operations consisted of opening the neck, pushing aside the pharynx, and drilling a hole in the usual spheno-occipital region of the skull without completely penetrating the bone.

Experimental tests were started on the 46th post-operative day, with the exception of one series which was started on the 14th post-operative day. In the cold ex-

periments the animals were kept at 5 to 6° C. for 4, 7, or 14 days. At the end of the experiments all animals were killed by decapitation and the thyroid, adrenals, gonads and seminal vesicles were weighed and fixed in 10% formalin for histological studies. The skulls were decalcified in 5% trichloracetic acid and the hypophysio-hypothalamic region cut serially to check the completeness of stalk section. All sections were stained with hematoxylin and eosin.

The cell height index (CHI) was used as an indicator of thyroid function. This index is the average cell height of 100 thyroid follicles (13). A somewhat similar cell height method has previously been used by Uhlenhuth (14) and Rawson and Starr (15) and is considered to be a sensitive indicator of thyrotropic hormone.

RESULTS

A. Changes in the pituitary stalk and hypophysis after stalk section. At the site of the lesion there appeared some hydropic degeneration with hypercellularity at the borders of the cut. Later the nervous tissue at the site of the lesion was resorbed and replaced by scar tissue. Degenerative changes were observed some distance proximally from the lesion (fig. 1).

The neural lobe showed similar changes, resulting in an appreciable decrease in size and occasionally in almost complete atrophy. The cells first became hydropic and later shrunken and atrophied. After incomplete section variable areas of normal tissue remained.

The pars intermedia was usually wrinkled and pushed into the residual lumen. The cells were hypertrophied and many of them were basophilic.

The anterior lobe showed variable changes. Sometimes after complete section no histological changes could be noted. Every gland showed large numbers of normal cells, but the eosinophiles tended to stain lighter than normally, apparently because of a loss of granules. These cells were often smaller than in controls. Numerous normal basophiles were always present, but there was a distinct tendency for large partially vacuolated basophiles to appear. Many showed the negative image of the Golgi apparatus (fig. 2). These changes were often present after incomplete section as well, but never after blank control operations.

Although complete stalk section should interrupt to some extent the hypophyseal portal venules and the superior hypophyseal arteries, no histological evidence of decreased circulation in the gland could be detected.

B. Thyroid. The results of 20 complete and 3 incomplete stalk sections were compared with 34 blank operation controls. The thyroid was studied, using the CHI method: (*a*) in operative controls; (*b*) in operative controls after exposure to cold; (*c*) after pituitary stalk section and (*d*) after pituitary stalk section and exposure to cold. The numbers of rats used in each experiment are given in table 1.

In the first series (A) the rats were placed in the cold (5 to 6° C.) for 7 days, beginning on the 46th day after operation. On the 53d post-operative day all the rats were killed. The table shows the results. In animals kept at room temperature after stalk section, the average CHI was normal (+12.5%). In blank operation controls and in one animal where the pituitary stalk was incompletely cut, cold stimulation caused an average of 60% increase in CHI (fig. 4). However, upon stimulation by cold after complete stalk section, no increase in CHI (+5.5%) was observed (fig. 3).

In a second series of experiments (*B*), the CHI of rats which were kept at room temperature was also normal (+3.3%) on the 18th post-operative day. The results of cold experiments starting on the 14th post-operative day, and lasting 4 days, were similar to those obtained in the first series. Five blank operation controls gave a CHI of +88% after cold; 4 complete

TABLE 1

Treatment	Series	No. rats	Thyroid[1]		Adrenals		Testicles		Seminal vesicles	
			CHI[2] ave. and variation	Difference	Ave. wt.	Difference	Ave. wt.	Difference	Ave. wt.	Difference
				%	mg.	%	mg.	%	mg.	%
Operative controls. Room temperature	A	15	4.0 (3.1–7.3)	—	29.2	—	2419	—	336	—
	B	7	3.0 (2.1–4.1)	—	32.1	—	—	—	—	—
Pituitary stalk section. Room temperature	A	5	4.5 (3.6–5.5)	+12.5	27.0	−7.5	2392	−2.0	389	+15.7
	B	5	3.1 (2.3–3.9)	+3.3	33.6	+4.6	—	—	—	—
Blank control op. *A*, 7 days in cold *B*, 4 days in cold	A	7	6.2 (4.9–7.6)	+56.0	36.2	+24.0	2204	−8.9	306	−15.7
	B	5	5.1 (3.0–6.3)	+88.0	34.3	+6.8	—	—	—	—
Complete pituitary stalk sec. *A*, 7 days in cold *B*, 4 days in cold	A	6	4.2 (3.7–4.9)	+5.5	33.7	+15.4	2514	+3.1	355	+5.6
	B	4	2.5 (2.1–2.8)	−17.0	31.2	−2.9	—	—	—	—
Incomplete pituitary stalk sec. *A*, 7 days in cold *B*, 4 days in cold	A	1	6.2	+55.0	26.5	—	2340	—	584	—
	B	2	7.0 (6.4–7.6)	+133.0	33.5	+4.3	—	—	—	—

[1] The thyroid weights are not included because of the lack of differences.
[2] CHI equals the average cell height of one hundred thyroid follicles counted by routine way.

stalk section animals gave a CHI of −17%, whereas 2 rats with incomplete stalk section, subjected to the same period of cold, exhibited a CHI of +133%.

Two rats with completely cut pituitary stalks failed to show any thyroid reaction after cold stimulation starting on the 46th post-operative day and lasting 14 days. Two animals with incomplete stalk section gave a mild reaction which was comparable to that of controls. The thyroid weights were within normal limits in all experiments.

C. *Adrenals.* The reaction of the adrenal glands to cold was studied according to the same principles as that of thyroid and with the same material (table 1).

In series *A* (rats killed 53 days after stalk section) in animals living at room temperature, the average weight of the adrenals was 7.5% smaller than in operative controls. Seven days cold exposure caused a 24% average

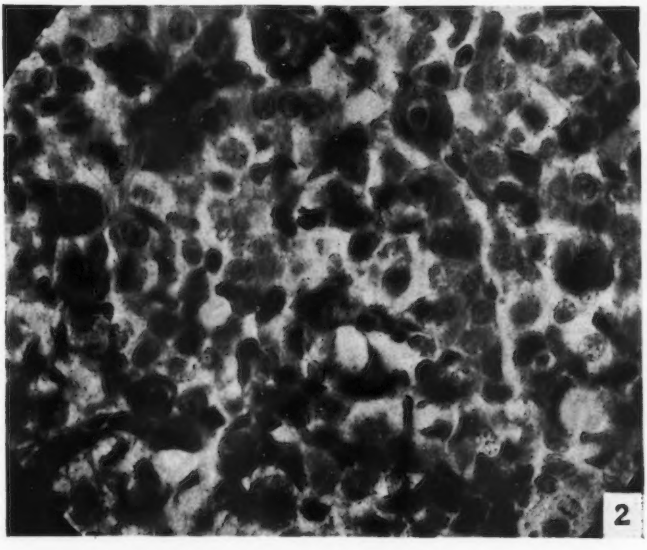

FIG. 1. THE INFUNDIBULAR REGION AND NEURAL LOBE AFTER COMPLETE STALK SECTION. Note the site of the section, the absence of damage to the hypothalamus, the degenerative changes in the infundibular stalk, and the atrophic changes in the neural lobe. Formol, hematoxylin and eosin. +125. Fig. 2. ANTERIOR PITUITARY AFTER STALK SECTION, SHOWING LARGE BASOPHILES, SOME OF THEM WITH VACUOLES AND NEGATIVE IMAGES OF THE GOLGI APPARATUS. Formol, hematoxylin and eosin. +600.

increase in the weight of adrenals in controls, whereas after stalk section plus cold stimulation the weight increase was 15%.

In series *B* (rats killed 18 days after the operation) the weights of the adrenals were normal (+4.6%). After 4 days of cold exposure the increase in the weight of the adrenals was minimal (+6.8%) in controls. After stalk section and cold exposure the adrenal weight remained normal (−2.9%). The same also was true after two incomplete stalk sections and cold stimulation (+1.8%). It was felt that in this series the cold stimulation was not

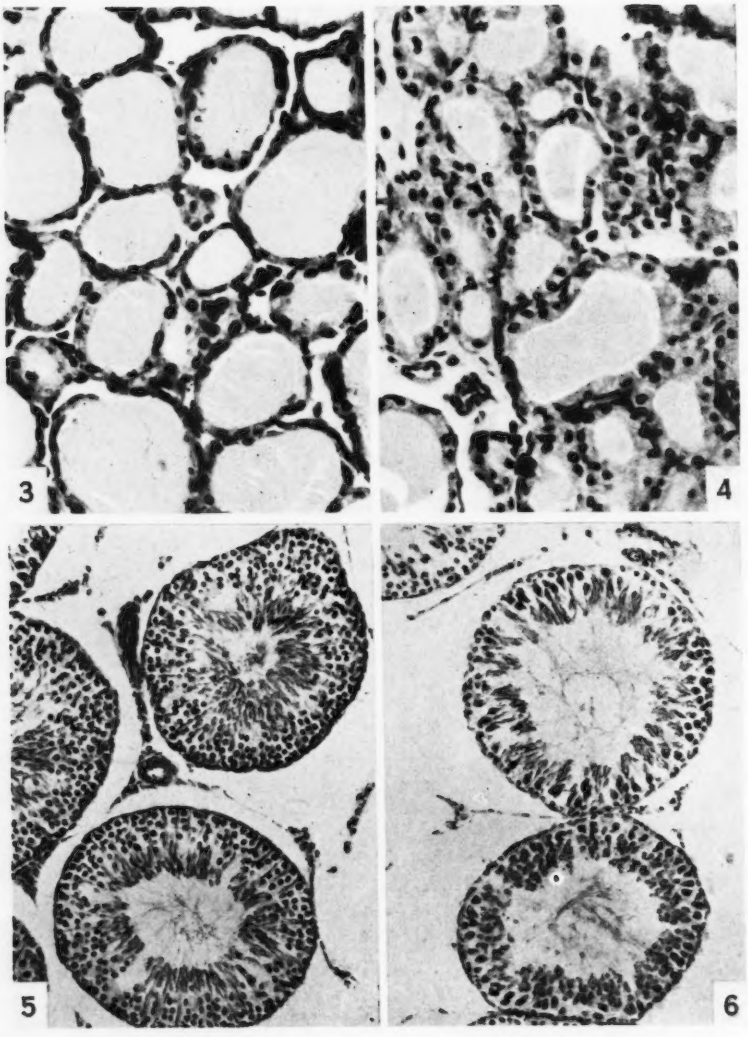

Fig. 3. THYROID AFTER PITUITARY SECTION AND 7-DAY COLD EXPOSURE. CHI = 3.5, a normal value Formol, hematoxylin and eosin. ×300. Fig. 4. THYROID AFTER 7-DAY COLD EXPOSURE IN BLANK OPERATION CONTROL. CHI = 6.2, 155% above normal average. Formol, hematoxylin and eosin. ×300. Fig. 5. TESTICULAR TUBULES AFTER STALK SECTION AND 7-DAY EXPOSURE TO COLD. Tubules are normal. Formol, hematoxylin and eosin. ×130. Fig. 6. TESTICULAR TUBULES AFTER 7-DAY COLD EXPOSURE IN BLANK OPERATION CONTROL. Note degenerative changes in layer of spermatocytes. Formol, hematoxylin and eosin. ×130.

strong enough because the controls also failed to react with adrenal hypertrophy. This happened in spite of the fact that the thyroids showed a marked cold reaction.

Histological study showed the adrenals to be normal after pituitary stalk section alone. If the animals were subsequently subjected to cold, with resulting adrenal weight increase, cortical hypertrophy was seen.

In some animals which became infected and which are not included in this material, the adrenal hypertrophy was apparent after complete stalk section even if no cold had been applied.

D. Testicles and seminal vesicles. The changes in the testicles and seminal vesicles were studied (*a*) in controls at room temperature; (*b*) after stalk section alone; (*c*) after 7 and 14 days cold exposure; and (*d*) after stalk section and similar cold exposure. The experiments were started 46 days after operation. The number of rats and the weights of the testicles and seminal vesicles are recorded in table 1.

After complete pituitary stalk section, the weights of the testicles and seminal vesicles were normal. Histologically no abnormalities could be detected in testicular tubules, interstitial cells or seminal vesicles (cf. fig. 5).

After 7 days of cold treatment the testicular weights of operative controls were 8.9% smaller and the weights of seminal vesicles 15.7% smaller than the corresponding weights of animals living at room temperature. Upon histological examination it was noted that, although every testicle had numerous normal tubules, there were definite degenerative changes in some. The spermatogonia usually showed no degeneration, the principal disorganization being present in the layer of spermatocytes. These were separated from each other, their number was decreased and the formation of spermatids had ceased. Pyknotic nuclei were found. Swollen, vacuolated and poorly staining cell debris was present in the lumen (fig. 6). The cold exposure did not cause histological changes in the seminal vesicles.

These changes in weight and microscopic appearance were much less marked or even totally absent in cases of cold exposure after complete stalk section (fig. 5). Only slight changes were found in one case of incomplete section.

Similar results were noted in a small series (2 complete and 2 incomplete stalk sections with controls) after 14 days in the cold, although the effect of cold in controls was less marked or even absent.

E. Growth. Growth measurements were recorded from 20 animals, 8 being operative controls, 8 complete stalk sections, and 4 incomplete stalk sections.

Figure 7 shows the average growth curves of these animals. After complete stalk section, growth was delayed only slightly (curve B) as compared with operative controls (curve A). However, 40 to 50 days after the operation, this difference disappeared.

After incomplete section (curve C) there was a slight tendency toward increased weight although the testicles were normal. The number of animals was too small to show whether the tendency to increased weight or adiposity was a characteristic of incomplete stalk section. In cases which developed a general pituitary insufficiency, growth was interrupted.

DISCUSSION

The cell changes in the anterior pituitary. The decreased acidophilia and the appearance of large, partially vacuolated basophiles after stalk section have been found previously in rabbits after the same operation (9). Similar

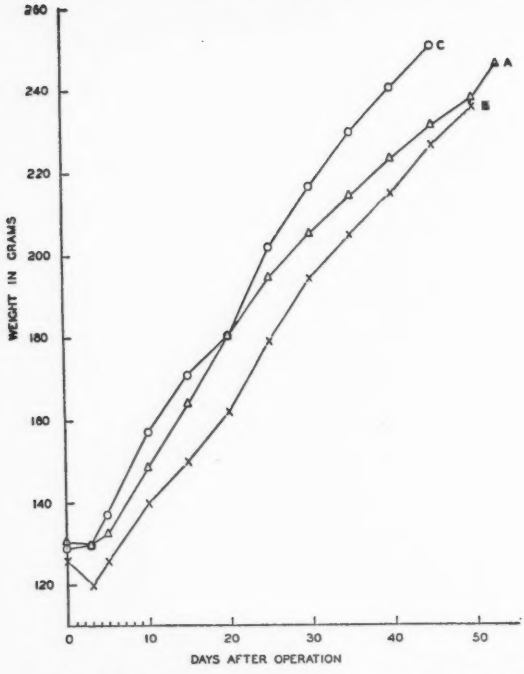

Fig. 7. GROWTH CURVES OF RATS AFTER PITUITARY STALK SECTION. Curve A = growth of operative controls. Curve B = growth after complete pituitary stalk section, Curve C = growth after incomplete pituitary stalk section.

changes have also been observed in thyroidectomized and castrated animals (for references see 16).

Pituitary stalk and thyroid. In agreement with Brooks (9) and Westman and Jacobsohn (11), it was found in the present study that the thyroid of rats is histologically normal after complete stalk section. This means that after the stalk operation the rats produce enough thyrotropic hormone to keep the thyroid histologically normal. Nevertheless, as demonstrated in the present experiments, stalk section, followed by exposure to cold, prevents the increased secretion of thyrotropic hormone with the subsequent thyroid hyperplasia ordinarily caused by cold. The lack of thyrotropic cold reaction after stalk section cannot be due to a general pituitary insufficiency since in the same animals the gonads are normal and the adrenals react with hyperplasia. From these facts one can conclude that the thermostatic function of the hypothalamus involves the thyrotropic hormone and thyroid function through the pituitary stalk when the animal is subjected to prolonged cold.

Moreover, these findings, taken together with the observations on the rôle of the cervical sympathetics in the regulation of thyrotropic hormone (13), indicate that the part played by the hypothalamus in the regulation of the thyrotropic hormone is much more important than that played by the cervical sympathetics.

Pituitary stalk and adrenal glands. After complete stalk section the adrenals of rats remain normal in weight and histology, thus confirming Brooks (9) and Westman and Jacobsohn (11). The new evidence presented in this study indicates that the adrenals of rats react with hyperplasia to cold after stalk section. Hence the regulation of the corticotropic function seems to be different from that of the thyrotropic hormone and does not depend on any direct hypothalamic control. Cervical sympathectomy also fails to affect the corticotropic function (13). It is therefore possible that the corticotropic hormone is under a humoral regulation.

Pituitary stalk and testicles. In agreement with Brooks (9) it was found that after a complete pituitary stalk section the testicles and seminal vesicles remain normal.

Pituitary stalk section in rats significantly decreases or completely abolishes the damage to the seminiferous tubules usually produced by exposure to cold. The direct effect of cold on the testes cannot be the explanation for this difference in reaction, since the testes themselves in equivalent experiments are presumably equally exposed to cold regardless of whether the pituitary stalk is severed or is intact. Hence one must assume that in normal animals subjected to cold, this stimulus acts through the pituitary stalk, depressing the production of gonadotropic hormones. This depressing effect of cold is abolished by section of the pituitary stalk.

Pituitary stalk and growth. The slight temporary retardation of growth after stalk section, as compared with blank operation controls, is of doubtful biological significance, since in every stalk operation the anterior pituitary is probably injured to a slight extent. This retardation of growth is in marked contrast to the tendency to weight increase noted after incomplete stalk section.

On the rôle of the pituitary stalk in the regulation of the anterior pituitary. The experimental data in the present paper, as well as the data published by Brooks (9) and Dempsey (10), indicate that the pituitary stalk pathways, under ordinary conditions, are not absolutely necessary for anterior pituitary function. This is evidenced by the maintenance of normal weights and histological structure in the other endocrines in spite of the appearance of some cytological changes in the anterior pituitary after stalk section. It is also evidenced by the fairly normal sexual behavior of hypophysectomized rats which have functioning pituitary grafts (17). This suggests that the anterior pituitary has a basic secretory rhythm which is independent of the pituitary stalk. After stalk section humoral agents can still alter this basic rhythm, as is evidenced by the fact that the ovaries react normally to prolan (9, 18), and that the testicles atrophy after stalk section and estrin treatment (19).

But although the pathways of the pituitary stalk are apparently unnecessary for the maintenance of anterior pituitary function under ordinary conditions, the available evidence points to their participation in certain specific adjustments to certain environmental situations. Amongst such specific stimuli mediated through the stalk one must include the ovulation response to mating in rabbits and the thyrotropic response to cold. The estrous

reaction to light of certain birds and mammals may be mediated in a similar way, although the experimental proof for this has not yet been forthcoming.

SUMMARY AND CONCLUSIONS

After pituitary stalk section in rats, enough thyrotropic hormone is produced to keep the thyroid histologically normal under ordinary conditions. However, stalk section, followed by exposure to cold, prevents the increased secretion of thyrotropic hormone and thyroid hypertrophy which normally occur in intact animals on exposure to cold. This negative effect is not due to a general pituitary insufficiency. Hence, it follows that in animals exposed to prolonged cold the hypothalamus stimulates the secretion of thyrotropic hormone by impulses transmitted through the pituitary stalk. The rôle of the pituitary stalk in this regulation is more important than that of the cervical sympathetics.

After pituitary stalk section in rats, as after cervical sympathectomy, the adrenals remain normal and react normally to cold with hypertrophy. Corticotropic secretion is hence probably under humoral control.

After pituitary stalk section in rats, the testicles and seminal vesicles remain normal, but the usual damaging effect of cold on spermatogenesis is abolished. Hence the influence of temperature on the secretion of gonadotropic hormones must be to a certain extent under hypothalamic control.

Stalk section does not significantly affect the growth of rats.

The anterior pituitary appears to have a basic secretory rhythm which is independent of the pituitary stalk under ordinary conditions. This rhythm is largely under humoral influences. Nevertheless, available evidence indicates that the hypothalamus and pituitary stalk participate in the regulation of anterior pituitary secretion in specific adjustments to certain environmental situations. In the present experiments, for example, evidence is adduced indicating the mediation of stimuli through the stalk upon prolonged exposure of the animal to cold.

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REFERENCES

1. Paulesco, N. C.: L'Hypophyse du Cerveau. Paris, 1908.
2. Bell, W. B.: The Pituitary. New York, 1919.
3. Dott, N. M.: *Quart. J. Exper. Physiol.* 13: 241. 1923.
4. Harris, G. W.: *Proc. Roy. Soc. London. s. B.* 122: 374. 1937.
5. Hinsey, J. C.: *Cold Spring Harbor Symp. Quant. Biol.* 5: 269. 1937.
6. Mahoney, W., and D. Sheehan: *Brain* 59: 61. 1936.
7. Westman, A., and D. Jacobsohn: *Acta Obst. et Gynec. Scand.* 17: 235. 1937.
8. Westman, A., and D. Jacobsohn: *Acta path. et microbiol. Scand.* 15: 301. 1938.
9. Brooks, C. M.: *Am. J. Physiol.* 121: 157. 1938.
10. Dempsey, E. W.: *Am. J. Physiol.* 1939 (in press).
11. Westman, A., and D. Jacobsohn: *Acta path. et microbiol. Scand.* 15: 435. 1938.
12. Swann, H. G., and P. E. Johnson: *Endocrinology* 24: 397. 1939.
13. Uotila, U. U.: *Endocrinology* 25: 63, 1939.
14. Uhlenhuth, E.: *Tr. Am. A. Study Goiter.* 1936.
15. Rawson, R. W., and P. Starr: *Arch. Int. Med.* 61: 726. 1938.
16. Severinghaus, A. E.: Allen's Sex and Internal Secretions. Baltimore, 1939.
17. Greep, R. O.: *Proc. Soc. Exper. Biol. & Med.* 34: 754. 1936.
18. Westman, A., and D. Jacobsohn: *Acta Obst. et Gynec. Scand.* 18: 109. 1938.
19. Uotila, U. U.: Unpublished data.

THE EFFECT OF ADAPTATION TO VARIOUS DAMAGING AGENTS ON THE FEMALE SEX ORGANS IN THE RAT

HANS SELYE

From the Department of Anatomy, McGill University

MONTREAL, CANADA

THE FACT that any change in surroundings or any unusual strain, such as violent muscular exercise, chronic diseases and even mental stress may disturb the sexual cycle of the female is well known from human pathology. Animal experiments confirmed these facts and countless publications have appeared in which the authors show how dietary deficiencies and intoxications may affect the female sex organs. In most cases, however, the authors who studied this phenomenon expressed the belief that the particular experimental intervention which they studied exerts a specific influence on the ovary or the sexual cycle. It is not within the scope of this publication to discuss the extensive literature on this subject in detail, but a few examples may help to illustrate our problem.

Insufficient food intake has been shown to cause irregularities of the cycle or permanent anestrus in the guinea pig (1, 2) and rat (3–5). In women, permanent amenorrhea, the so-called Kriegsamenorrhoe of the German authors, was frequently seen among the starving population of central European countries during the World War (6).

Excessive quantities of beans (*Phaseolus vulgaris*) if given with the diet may considerably interfere with the estrous cycle of the mouse (7, 9). Rats fed wholly on boiled beef muscle grow at a practically normal rate, but in most cases, their first estrous cycle is delayed and subsequent cycles are infrequent (10). Deficiency in vitamin A (11), vitamin B (5, 10, 12, 13), vitamin C (14) or vitamin D (15) and other qualitative nutritional deficiencies have also been shown to cause disturbances of ovarian function and suppression of the cycle.

Muscular exercise has often been found to cause menstrual irregularities in women (16, 19).

Various drugs such as choline (20), atropine (21,–23), caffeine (24), codeine (25), eserine (22), morphine (25, 26), nicotine (27, 28) etc., may likewise exert an inhibitory effect on ovarian function in women and experimental animals. Most of the investigators who observed ovarian or estrous changes following treatment with stimuli such as those mentioned above believed that the action on the sex organs is due to the specific pharmacological action of the drugs used or to some specific effect of the employed experimental procedure. However, it is difficult to believe that such entirely different stimuli should all have similar specific effects. It seems much more likely that there is some common pathway through which these stimuli influence the gonads. Selye and Collip (29) expressed the opinion that under the influence of

damaging agents, there may be a 'shift' in the hormone production of the pituitary, since the same agents which cause atrophy of the sex glands and anestrus in the female, usually also elicit enlargement of the adrenal cortex and inhibition of growth. It is conceivable that in conditions of emergency the pituitary would produce more adrenotropic and less gonadotropic and growth hormone. On the other hand, Mulinos et al. (30) expressed the opinion that since loss of weight is common in all these cases of non-specific estrus inhibition, the latter is "either wholly or at least in part due to the concomitant inanition." It is noteworthy, however, that the loss of weight is not necessarily an accompaniment of non-specific estrus inhibition, since the above mentioned experiments of Evans and Bishop (10) showed that rats may grow at an approximately normal rate on a boiled beef muscle diet and yet show marked inhibition of estrus. Furthermore Hammond and Marshall (31) noted estrus inhibition in cows which became particularly fat as a result of excessive food intake and forced feeding of geese leads to ovarian atrophy in spite of the considerable fattening caused by this procedure. These observations are difficult to explain in the light of the inanition theory.

It occurred to us that perhaps the inhibition of estrus might be a direct result of the organism's effort to adapt itself to changed environmental conditions. A systematic study of the somatic changes during adaptation to various stimuli has shown that following exposure to damaging agents, a syndrome occurs whose symptoms are always the same irrespective of the nature of the damaging agent which elicited them. This has been termed 'the general adaptation syndrome.' It has been noted that when considerable adaptation is acquired to such different agents as excessive muscular exercise or cold or intoxication with various drugs, the adrenals are usually large, the gonads small, the hematocrit values low, there is hyperglycemia, hyperchloremia and hypophosphatemia (32–36). All these changes have been regarded as entirely independent of the specific nature of the agent which produced them and as in some way connected with the acquisition of adaptation as such. So little is known about the mechanism of adaptation that it is difficult to understand the significance of the changes observed during this general adaptation syndrome. The only reason for considering them as parts of one reaction is that they usually appear together. While a high degree of adaptation to a certain agent is acquired, the resistance of the organism to agents of a different nature decreases below normal (37). This gives the impression that the organism expends some 'adaptation energy' during the acquisition of inurement and consequently while its resistance to one agent rises, it loses some of its adaptability to other stimuli. It is conceivable that in the case of non-specific estrus inhibition, we are dealing with a similar phenomenon insofar as the organism may not be able to maintain its normal sexual function, if at the same time, it has to acquire adaptation to important changes in its internal or external environment. If this interpretation should prove to be correct, one would expect the estrous cycles to reappear in spite of changed external conditions as soon as the organism acquired complete adaptation to the change. In order to test this hypothesis, the following experiments were performed.

EXPERIMENTAL PROCEDURE AND RESULTS

Thirty adult 'hooded' female rats weighing 157 to 220 gm. were divided into 5 groups. Six animals were forced to exercise 15 minutes 3 times daily in drum cages having a diameter of 12 inches and revolving at a speed of 18 to 22 revolutions per minute. Their daily exercise periods were gradually increased to 3 times 60 minutes daily within a month and they were maintained at this level of exercise during the rest of the observation period. Six other rats of this series received ground white lima beans mixed in with their ground Purina diet in an amount corresponding to 25% of the total food consumed. The percentage of lima beans in this diet was gradually increased within 7 weeks to 60% of the food intake and the animals received a mixture of 40% Purina and 60% lima beans during the rest of the experiment. Another group of 6 rats was treated with 0.2 cc. of a 2% morphine sulphate solution daily throughout the experiment without changing the dose. While all the previously mentioned rats received as much food as they wanted, the last experimental group of 6 rats was given only 10 gm. of ground Purina per rat daily during the first few days and then the food intake was gradually further decreased until 6 weeks after the beginning of the treatment, they only received 6.5 gm. per rat daily. The remaining 6 animals served as untreated controls. All rats of this series were known to have had normal estrous cycles during the last 2 weeks before initiation of the experiment and their vaginal contents were examined daily during the entire length of the experiment which lasted 3 months.

It was observed that the vaginal estrous cycles continued approximately normal throughout the whole experiment in all groups except the morphine-treated series in which a transitory anestrous period of about one month developed soon after initiation of the treatment. In the other groups, slight irregularities of the vaginal estrous cycles were occasionally noted during the first few days after increasing the dose of the stimulus with which they were treated. However, no serious disturbances were observed and the ovaries of the animals killed at the end of the observation period showed no significant deviations from those of the normal control group. At the end of the experiment, the body weight of the animals in the exercised group and of those receiving beans was higher than at the initiation of treatment while in the morphine-treated group and especially in the animals receiving the quantitatively insufficient diet, there was a marked loss of weight. Yet during the last weeks of the experiment, the estrous cycles were practically normal, and at autopsy the ovaries showed no change. It may be concluded that if adaptation is gradual, the sex organs are not necessarily damaged by the above mentioned agents. In the case of morphine where the initial dose was relatively high, there was a transitory diestrous period which, however, gave place to normal estrous cycles after the animals became sufficiently adapted to the drug.

It appeared important to prove that in the above mentioned experiments, the maintenance of normal estrous cycles in the groups other than the

morphine-treated series was due to the fact that adaptation was gradual. To do this, we had to show that exposure to such doses of the various agents as were given during the last few weeks of the previous experiment would cause cessation of the cycles, if not preceded by a gradual adaptation period. For this purpose, 24 adult female 'hooded' rats of approximately the same size as those used in the first experiment were divided into 4 groups. Six of them received a ground Purina diet containing 50% ground white lima beans. Six rats were forced to exercise for 60 minutes, 3 times a day under the same conditions as the animals of the first series. Six rats received 7 gm. of ground Purina per animal daily. The remaining 6 rats were not treated with morphine as in the previous experiment, since it was obvious from the above mentioned findings that the dose of this drug which was compatible with the maintenance of normal sexual cycles in pretreated animals caused prolonged anestrus during the first weeks of treatment. But in the case of morphine, adaptation might be regarded as specific to this drug to which, as is generally known, a particularly high degree of adaptation may be acquired. We therefore decided to treat the last group with formaldehyde which is not a habituation drug but which, as previous experiments have shown, elicits a typical 'general adaptation syndrome.' With this in mind, we administered 0.2 cc. of a 4% formaldehyde solution, 3 times daily to the rats of this last group. In this experimental series, we also showed by the vaginal smear technic that all animals exhibited normal cycles during the last 2 weeks before initiation of treatment. Within the first 6 days, all animals in the various groups of this series became permanently anestrous with the exception of one rat in the lima bean fed group which maintained normal cycles throughout the experiment. This, however, is very exceptional and numerous unpublished experiments performed in our laboratory indicate that continuous anestrus is regularly obtained in rats receiving a diet containing 40% or more lima beans.

All animals were killed 40 days after initiation of treatment and both their adrenals and ovaries were weighed and histologically examined (table 1).

It appears from the data of table 1 that all 4 types of treatment led to a marked decrease in ovarian weight. This was accompanied by an increase in the average adrenal weight in all but the lima bean fed group. The rats of this group and a few among the animals receiving the insufficient diet had adrenals which were below normal in weight. Such a decrease in adrenal weight has sometimes been noted in our previous experiments on adaptation. It is usually seen after an initial period of adrenal hypertrophy in animals treated with relatively mild damaging agents. The mechanism responsible for the production of the adrenal enlargement and the secondary adrenal atrophy which sometimes follows, is not known as yet. Microscopic studies show, however, that both these reactions are due to changes in the adrenal cortex while the medulla remains relatively intact (33).

Histological examination of the ovaries shows that the decrease in weight is due first of all to the scarcity of mature follicles and corpora lutea and secondly to marked involution of the interstitial tissue. However, we

found it surprising that even though all animals showed continuous anestrus for many weeks before being killed, fresh corpora lutea and mature follicles were observed in many cases. Figure 1 shows a large mature follicle in the ovary of a formaldehyde-treated rat which exhibited no signs of vaginal

TABLE 1. DECREASE IN OVARIAN WEIGHT AND THE ADRENAL WEIGHT CHANGES DURING THE GENERAL ADAPTATION SYNDROME

		Ovaries	Adrenals
Untreated controls	1	41	62
	2	60	76
	3	41	58
	4	42	72
	5	83	70
	6	54	71
	7	39	50
	Average	51.4	65.6
Lima beans	1	15.1	48
	2	10.1	46
	3	14	62
	4	16	50
	5	34.2	47
	6	25	43
	Average	19.1	49.3
Formaldehyde	1	12.1	144
	2	22.1	155
	3	30.2	156
	4	22.2	74
	5	23.1	150
	6	15.6	127
	Average	20.9	129.3
Exercise	1	25.0	100
	2	24.0	126
	3	24.0	100
	4	26.2	106
	5	24.2	108
	6	34.2	70
	Average	26.3	101.7
Insufficient diet	1	20.1	100
	2	34.0	90
	3	29.2	60
	4	14.1	90
	5	15.2	64
	6	18.2	44
	Average	21.8	74.7

estrus. It was striking, however, that instead of the usual set of about 6 to 15 mature follicles or active corpora lutea, only 1 or 2 follicles or apparently functional corpora lutea were seen in most of the animals. Most of the corpora lutea showed marked signs of involution and were embedded in atrophic interstitial tissue. The theca cells surrounding the numerous atretic follicles in such ovaries and those of the theca nests showed typical trans-

formations into so-called wheel cells described by Selye et al. (38) as characteristic signs of hypophyseal deficiency (fig. 2, 3, 4). Pomerantz and Mulinos (39) described the appearance of such cells following inanition and regarded them as a sign of 'functional pseudohypophysectomy.' They believed that inanition decreases the function of the pituitary and that this

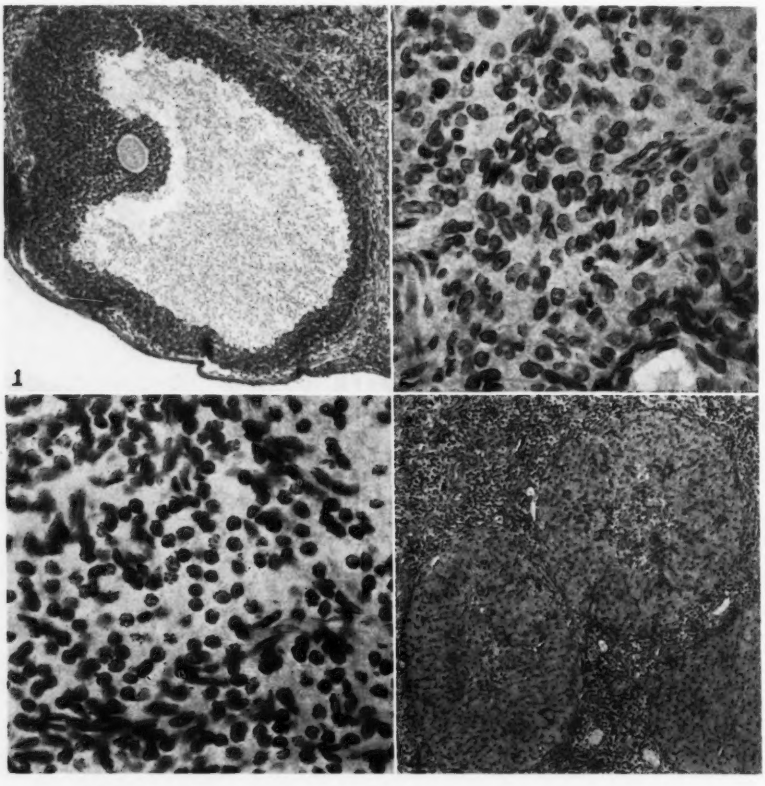


Fig. 1. HIGH MAGNIFICATION OF MATURE GRAAFIAN FOLLICLES IN THE OVARY OF A FORMALDEHYDE-TREATED RAT.

Fig. 2. INTERSTITIAL TISSUE OF A NORMAL RAT OVARY UNDER HIGH MAGNIFICATION.

Fig. 3. INTERSTITIAL TISSUE OF THE OVARY IN A FORMALDEHYDE-TREATED RAT UNDER THE SAME MAGNIFICATION AS FIG. 2. Note the atrophic cells and the transformation of the neuclei into wheel-like structures.

Fig. 4. LOW MAGNIFICATION OF THE OVARY OF A FORMALDEHYDE-TREATED RAT. Note the involuting corpora lutea surrounded by atrophic interstitial tissue.

explains why non-specific damaging agents which cause inanition also cause atrophy of the sex organs. Nevertheless, the reasons enumerated in the introductory chapter of this communication as well as our above mentioned observations, showing that non-specific estrus inhibition is not necessarily combined with loss of body weight, are difficult to reconcile with such an interpretation. The fact that ovarian atrophy caused by non-specific damage

is often accompanied by marked adrenal enlargement; as shown in our formaldehyde, exercise and quantitatively insufficient diet series; indicates furthermore, that the adrenotropic hormone production of the pituitary is not inhibited at a time when the ovary shows obvious signs of damage. These findings appear to confirm the view that during adaptation to non-specific damaging agents, there is no general decrease in hypophyseal activity but there tends to be a shift in the hormone production of the gland. As a result of this, the pituitary produces more corticotropic and less gonadotropic hormone. These findings are in good agreement with the assumption that the involution of the gonads and the cessation of the sexual cycle caused by non-specific damaging agents, represent part of the defence reaction of the organism against damage and that they may be regarded as symptoms of the 'general adaptation syndrome.'

The fact that follicle maturation and corpus luteum formation are not necessarily prevented during non-specific estrus inhibition required special consideration. There is no doubt that in our treated animals follicles matured fully and corpora lutea formed without there being any sign of estrus as judged by the vaginal smear technic. Thus the vagina of the animal whose ovary contained the mature follicle shown in figure 1 was mucified and exhibited no trace of cornification. Such a dissociation between the ovarian cycle and the periodic changes in the other female sex organs have already been noticed by several previous investigators. Thus Parkes (40, 41) showed that in mice after x-ray sterilization, uterine and vaginal estrous cycles may continue although follicle maturation and corpus luteum formation no longer occur. In *Macacca mulatta* menstruation or at least periodic uterine bleeding resembling menstruation, may be observed without there being corpus luteum formation or ovulation (42, 43) and the same is true, though less frequently, in the human female (42, 44).

The reverse condition, that is to say, ovulation without external signs of estrus as described in the present communication, should likewise not be regarded as entirely impossible. In the bat (*Vesperugo pipistrellus*), ovulation occurs during March or April, while external signs of estrus and mating occur in September and October and thus the spermia have to wait in the genital organs of the female several months before the ovum is ready for them (45, 46). In this species, therefore, a dissociation between the external signs of estrus and ovulation appears to be a physiological phenomenon. In ewes, treated with gonadotropic hormone, ovulation may be produced without there being estrous behavior or any sign of vaginal estrus. Yet under such conditions, ovulation seems to give rise to fertile ova since forced mating may result in pregnancy (47, 48). In rats maintained on a lysine deficient diet, Lafon and Veillet (49) observed continuous vaginal anestrus although the ovarian cycles proceeded normally. These investigators believe that under the influence of the lysine deficient diet the vaginal epithelium becomes relatively irresponsive to estrin; however, they performed no experiments to substantiate this view. In order to check their assumption, the following experiment was performed.

Twenty-four adult 'hooded' female rats weighing between 165 and 227 gm. were ovariectomized. Beginning one week after the operation, 6 of these animals received 0.2 cc. of a 4% formaldehyde solution 3 times daily, 6 were forced to run for 60 minutes 3 times daily as in the previous experiment, and 6 were placed on a diet of 7 gm. of ground Purina daily per animal. The remaining 6 ovariectomized rats served as controls. All 24 animals received 30γ of estradiol in 0.1 cc. of Mazola oil during the first 3 days and all of them showed vaginal cornification after the third injection. From then on the dose of estradiol was decreased 5γ every 3 days in order to establish the minimum amount capable of maintaining vaginal estrus in these animals. It was found that the cornified cells began to disappear from the vaginal contents at the same time in all groups, that is, when the dose reached the level of 5γ per rat. Since the same or even a slightly smaller dose of the damaging agents to which the 3 groups of treated rats were exposed, sufficed to produce continuous anestrus in normal females, as reported in an earlier section of this paper, it appears that the estrus abnormality cannot be regarded as due to a decreased estrin sensitivity of the vaginal epithelium. These findings

TABLE 2. EFFECT OF NON-SPECIFIC DAMAGE ON THE GONADOTROPIC ACTIVITY OF APL IN RATS. THE OVARIAN WEIGHTS ARE GIVEN IN MG.

APL	110	105	97	56[1]	69	100	Average 96.2
APL and Formaldehyde	85	55	98	59	68	56	Average 70.2

[1] This animal suffered from chronic peritoneal infection so that it really should be regarded as an example of the effect of APL as influenced by non-specific damage. It has therefore, been eliminated from the average.

are in agreement with those of Mulinos et al. (30) who without attempting to compare the responsiveness of damaged animals with that of normals, found that estradiol benzoate may cause vaginal estrus in non-castrate females damaged by inanition.

Furthermore it appeared of interest to establish if the ovarian atrophy which occurs during the general adaptation syndrome is due to loss of sensitivity of the gonad to the gonadotropic hormones of the pituitary. In order to test this possibility 12 adult female 'hooded' rats weighing 178 to 222 gm. were treated with 10 R.U. of urinary gonadotropic hormone (APL) for 4 days and were killed on the day after the last injection. Six of these rats had received 0.2 cc. of a 4% formaldehyde solution 3 times daily for 12 days prior to and during the 4 days of the gonadotropic hormone treatment. The following table shows the ovarian weights in the 2 groups.

It is evident from this table and from similar experiments performed in our laboratory which we do not report here, that, although the ovaries undergo marked atrophy during the adaptation syndrome, they do not become insensitive to injected gonadotropic hormones. However their response is slightly less marked than that of normal ovaries. It is conceivable that this is partly due to the decrease in the gonadotropic hormone production of the animals' own pituitaries which, as we now know, synergizes the effect

of injected gonadotropic preparations. The fact that external conditions may influence the response to pituitary principles should be kept in mind when gonadotropic hormone assays are evaluated. At the same time, it must be emphasized that if very large doses of urinary gonadotropic hormones are administered, the difference between the responsiveness of the non-specifically damaged and the normal ovary may become negligible (29). In any case, we cannot speak of complete suppression of pituitary function since in hypophysectomized rats, APL leads only to theca luteinization (50–52) while in the above mentioned experiments, it caused corpus luteum formation.

SUMMARY

Experiments on the rat indicate that during exposure to non-specific damaging agents (such as excessive muscular exercise, qualitatively or quantitatively inadequate diets, or toxic doses of various drugs) the ovaries undergo atrophy and more or less permanent anestrus ensues. These changes cannot merely be due to loss of weight since, depending on the experimental conditions, anestrus may occur even though the body weight increases or vaginal cycles may remain normal in spite of loss of body weight.

The same dose of a damaging agent which causes ovarian atrophy and anestrus in a not pretreated rat fails to exert such an effect if it is administered following a period of pretreatment with gradually increasing doses. It has also been shown that the cycles' reappearance after adaptation is acquired to an agent which first inhibited vaginal estrus.

The changes in the female sex organs caused by non-specific damaging agents cannot be regarded as a result of a general depression of pituitary function since while the ovaries involute, the adrenal cortex usually exhibits marked hypertrophy indicating excess corticotropic hormone secretion. It is assumed that the atrophy of the sex organs is part of the 'general adaptation syndrome,' that is, of the non-specific reaction of the organism during adaptation to changes in its external or internal environment. It appears that in cases of emergency, the pituitary tends to produce more adrenotropic and less gonadotropic hormone than under normal conditions. The reason for this is probably that, under such conditions, an abundant supply of the life-maintaining principle of the adrenal cortex is a more imminent necessity than the preservation of normal sex function.

During the anestrus of the general adaptation syndrome the ovaries may develop just as severe an atrophy as after hypophysectomy and the 'wheel cells' characteristic of hypophyseal insufficiency may appear. On the other hand, even though the anestrus is permanent, follicle maturation and corpus luteum formation may occur in animals which are not especially severely damaged. Under these conditions, usually one or two follicles are transformed into corpora lutea at the same time. Apparently there exists a counterpart to the so-called 'anovular cycle.' This may be termed 'anestrus ovulation.' Certain observations reported in the literature indicate that such ovulations may also occur in women. They might be referred to as 'amenstrual ovulations.'

Experiments on ovariectomized rats indicate that the estrin sensitivity of the vagina is not significantly influenced by the general adaptation syndrome.

The sensitivity of the ovary to urinary gonadotropic hormone is but slightly decreased by non-specific damaging agents.

REFERENCES

1. Papanicolaou, G. N., and C. R. Stockard: *Proc. Soc. Exper. Biol. & Med.* 17: 143. 1920.
2. Zambonini, A., and P. Bortolucci: *Monit. ostetr. e. ginec.* 7: 65. 1935.
3. Loeb, L. *Biol. Bull.* 33: 187. 1917.
4. Long, J. A., and H. M. Evans: *Mem. of the Univ. of California* 6: 1. 1922.
5. Marrian, G. F., and A. S. Parkes: *Proc. roy. Soc. Lond.* 105: 248. 1929.
6. Fraenkel, L.: *Zentralbl. f. Gynäk.* No. 44 .1917.
7. Belak, S., and J. Szathmary: 5. Tagung der Ungar. Phys. Ges. Debrecen, 3–6, VI. 1935.
8. Belak, S., and J. Szathmary: *Biochem. Ztschr.* 291: 259. 1937.
9. Zselyonka, L., and A. Illényi: *Biochem. Ztschr.* 291: 263. 1937.
10. Evans, H. M., and K. S. Bishop: *J. Metabolic Res.* 1: 335. 1922.
11. Susaki, R.: *Kinki Fujinkwa Gakkwai Zassi* 9: 25. 1936.
12. Parkes, A. S.: *Quart. J. Exper. Physiol.* 18: 397. 1928.
13. Nakamura, M.: *Trans. jap. Path. Soc.* 19: 85. 1929.
14. Goettsch, M.: *Proc. Soc. Exper. Biol. & Med.* 27: 71. 1929.
15. Bischoff, H.: *Monatschr. f. Kinderheilk.* 73: 300. 1938.
16. Dybowska, J.: *Polska Gaz. lek.* 12: 17. 1933.
17. Kosakae, J., K. Kawanabe, Y. Kuraishi, and A. Yamamura: *Jap. J. Obstet. & Gynäk.* 16: 141. 1933.
18. Duntzer, E., and M. Hellendal: *Münch. med. Wschnschr.* 76: 1835. 1929.
19. Czarnocka-Karpinska, W.: *Wychowanie fizyczne* 17: 1. 1936.
20. Duncan, D. R. L., T. F. Gallagher, and F. C. Koch: *J. Biol. Chem.* 119: XXVII. 1937.
21. Mavromati, L.: *Compt. rend. Soc. de Biol.* 111: 191. 1932.
22. Foster, M. A., H. F. Haney and F. L. Hisaw: *Proc. Soc. Exper. Biol. & Med.* 32: 351. 1934.
23. Makepeace, A. W.: *Endocrinology* 23: 241. 1938.
24. Weiss, H.: *Arch. f. exper. Path. u. Pharmakol.* 186: 33. 1937.
25. Ko, K.: *J.M.A. Formosa* 33: 50. 1934.
26. Kraus, W. M.: *J. Nerv. & Ment. Dis.* 48: 36. 1918.
27. Unbehaun, G.: *Arch. f. Gynäk.* 147: 371. 1931.
28. Nakasawa, R.: *Jap. J. med. Sci., Trans. IV. Pharmacol.* 7: 1. 1933.
29. Selye, H., and J. B. Collip: *Endocrinology* 20: 667. 1936.
30. Mulinos, M. G., L. Pomerantz, J. Smelser and R. Kurzrok: *Proc. Soc. Exper. Biol. & Med.* 40: 79. 1939.
31. Hammond, J., and F. H. A. Marshall: *Proc. Roy. Soc. London* 105: 607. 1930.
32. Masson, G.: *Compt. rend. Soc. de Biol.* 130: 886. 1939.
33. Selye, H. *Endocrinology* 21: 169. 1937.
34. Selye, H.: *Arch. internat. de Pharm. et de Thérap.* 60: 259. 1938.
35. Masson, G., and H. Selye: *Compt. rend. Soc. de Biol.* 130: 1442. 1939.
36. Leblond, C. P., Nguyen-van Thoai and G. Segal: *Compt. rend. Soc. de Biol.* 130: 1557. 1939.
37. Selye, H.: *Am. J. Physiol.* 123: 758. 1938.
38. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* 17: 494. 1933.
39. Pomerantz, L., and M. G. Mulinos: *Proc. Am. Physiol. Soc.* Toronto (1939).
40. Parkes, A. S.: *Proc. Roy. Soc. London s.B.* 100: 172. 1926.
41. Parkes, A. S.: *Proc. Roy. Soc. London s.B.* 101: 421. 1927.
42. Hartman, C. G.: *Am. J. Obstet.* 19: 511. 1930.
43. Corner, G. W.: *Medicine* 12: 61. 1933.
44. Ogorek, M.: *Zentralbl. f. Gynäk.* 35: 1236. 1911.
45. Courrier, R.: *Compt. rend. Soc. de Biol.* 87: 1365. 1922.
46. Courrier, R.: *Compt. rend. Soc. de Biol.* 99: 26. 1928.
47. Cole, H. H., and R. F. Miller: *Am. J. Anat.* 57: 39. 1935.
48. Zawadowsky, M., P. Vunder, A. Padootsheva and S. Margvelashvili: *Trans. Dynam. Devel.* 9: 21. 1935.
49. Lafon, M., and A. Veillet: *Compt. rend. Soc. de Biol.* 129: 256. 1938.
50. Collip, J. B., D. L. Thomson and H. Selye. *J. Biol. Chem.* 100: XXXI. 1933.
51. Collip, J. B., H. Selye and D. L. Thomson: *Virchows Arch. f. path. Anat.* 290: 23. 1933.
52. Collip, J. B., H. Selye and D. L. Thomson: *Nature* 131: 56. 1933.

THE PART PLAYED BY AGE AND THE FEMALE SEX HORMONES IN RESISTANCE TO INFECTION

DOUGLAS H. SPRUNT

From the Department of Pathology, Duke University School of Medicine

DURHAM, NORTH CAROLINA

THAT THE ENDOCRINE pattern of the host has an effect on the host's response to disease agents has been appreciated since the work of Bullowa and Kaplan in 1903 (1). These workers then showed that patients with asthma improved when adrenalin was injected. Since this time there have been a number of papers both clinical and experimental pertaining to this subject. In this paper we do not intend to cover the whole field of the relationship of the endocrines to infection but only that portion which concerns age and the sex hormones. The literature on this subject falls naturally into three main groups—antibody formation, morphological changes and the dispersion and concentration of disease agents.

The effect of age and the female sex hormones on antibody response has been extensively studied. Baumgartner (2) in 1932 reviewed over 200 references on this subject and came to the conclusion that age increased the ability of the body to respond to artificial antigenic stimulation. Schultz (3) in 1934 added further substantiation to this conclusion from the literature and also showed experimentally that adult male rabbits were more easily sensitized to protein than were younger ones.

The increase in the number of negative Schick tests with age as reported by Von Gröer and Kassowitz (4) and also the findings of Schick negative individuals in Greenland where diphtheria is unknown (5) has led to a considerable discussion as to whether the host can develop antibodies to a disease without being exposed to it. This literature is well reviewed by Andrewes and associates (6).

Jungeblut and Engle (7) report that old monkeys as well as monkeys which have been injected with anterior pituitary extracts develop substances in their sera which are capable of neutralizing the virus of poliomyelitis As a result of this work they call in question the classic theory of immunology, that one must have at least a subclinical infection before one can develop antibodies to a disease. The work of Aycock (8) in which it was found that castrate monkeys given the estrogenic hormone were more resistant to infection with the poliomyelitis virus than ones which had not received this hormone lends some support to the contention of Jungeblut and Engle. At present, however, the data available do not warrant in either diphtheria

or poliomyelitis, one's saying that antibody can develop without exposure to the disease agent, but it does appear certain that older animals develop antibodies more readily than younger ones.

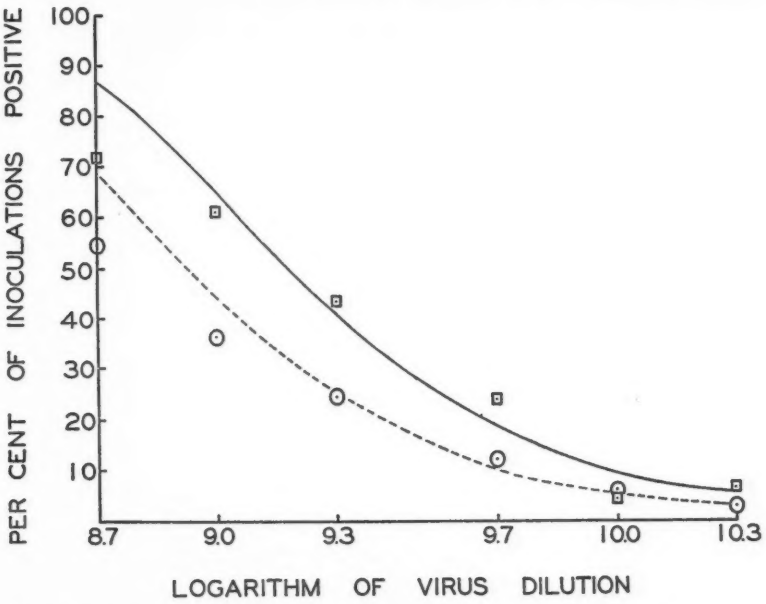
There have also been numerous reports on the effect of the sex hormones on allergic conditions. Ratnoff (9) recently reviewed these and reported experiments on anaphylactic shock in pregnant rats. His experiments showed that pregnancy had no bearing on the rat's susceptibility to anaphylaxis. And although some of the other workers modified allergic conditions with certain hormones there is still too much disagreement to draw any definite conclusions.

Increased resistance to infection has been attributed to morphological changes induced by the injection of certain sex hormones. Huberman and Israeloff (10) were able to cure gonococcal vaginitis in children by changing the type of mucosa in the vagina with the estrogenic hormone. Young and Marks (11) prevented peritonitis following laparotomies by the injection of amniotic fluid in the abdomen before operation. This material caused a mobilization of macrophages which led to the host's more easily overcoming bacterial contamination. Ziskin and his associates (12) have shown in several papers that pregnancy and estrogenic hormones produce a change in the mucosa of the mouth which makes the gums more resistant to infection.

The effect of age, pseudopregnancy, castration and the estrogenic hormone on the dispersion and concentration of disease agents in the skin and the bearing of this phenomenon on resistance to infection has been studied by the author's associates and himself. It was (13) found by the study of a number of rabbits that the spread of India ink varied in the following manner. The spread was smallest in female rabbits just after weaning. The male rabbits just after weaning, the pseudopregnant adult female rabbits, adult female rabbits, which had been castrated for less than 2 months and adult female rabbits which had been castrated and then given the estrogenic hormone showed a slight but statistically significant larger spread of the India ink. The adult male rabbits, the adult non-pseudopregnant female rabbit and castrate rabbits 5 to 6 months after castration had the largest dispersion of India ink.

Duran-Reynals (14) and his associates in their experiments with aqueous extracts of testis showed that the dispersion of vaccine virus paralleled the dispersion of India ink and found also that some disease agents such as a moderately virulent staphylococcus when dispersed produced no lesions as the spread of the organism made it possible for the natural resistant powers of the skin to overcome the scattered bacteria. We decided to follow up these experiments by a study of the effect of concentration and dispersion on the vaccine virus. We realized that if this could be done on a quantitative basis the results would be more significant. Parker (15) has recently described a statistical method by which it is possible to estimate the number of particles of vaccine virus which are required to cause infection in the rabbit skin. Our study of this method showed that it estimates the number of virus particles reaching susceptible cells and not the number in the inoculum necessary to

produce infection. By the use of this method it was found that there is always some factor or factors present in the skin of all rabbits which neutralizes a certain proportion of the virus injected. The estrogenic hormone and pseudopregnancy were shown to increase these factors. A typical experiment showing the effect of pseudopregnancy on the neutralization of virus is shown in the chart. The horizontal difference between the 2 curves is seen always to be constant. This difference can be shown mathematically to be always a constant proportion of the number of virus particles reaching susceptible cells in the control animals, and hence is a measure of the increase in resist-



GRAPH SHOWING INCREASED RESISTANCE OF PSEUDOPREGNANT RABBITS TO INFECTION WITH VACCINIA. Unbroken line—Adult male rabbits. Broken line—Pseudopregnant rabbits.

ance produced by pseudopregnancy and the estrogenic hormone. In 4 experiments in which we compared castrate rabbits with castrate rabbits receiving the estrogenic hormone we found that 33.4% of the virus; was neutralized by the estrogenic hormone. In 3 experiments in which we compared normal adult male rabbits with adult pseudopregnant rabbits we found 46.2% of the virus was inactivated by pseudopregnancy.

From these experiments it is seen that age and the sex endocrine pattern have an effect on the dispersion and concentration of disease agents in the skin. The concentration of vaccine virus is shown to be favorable to the host. It should be borne in mind, however, that concentration of the disease agent is not necessarily to the advantage of the host as Duran-Reynals (14) has shown that the dispersion of moderately virulent bacteria leads to their being

overcome by the host. We are at the present time studying the effect of concentration of certain fungi of low virulence. Although as yet we can say nothing definite, we believe that these organisms can only gain a foothold in the host when they are localized. This we believe will have a direct bearing on why certain skin diseases clear up at puberty, as it is at this time that the causative organisms would be dispersed over a larger area.

Although much more work is needed on the effect of the endocrine pattern on all three of these factors—antibody formation, morphological changes and dispersion and concentration, it is evident that they all are affected by the endocrine pattern of the host and that they all play a part in the host's resistance to disease agents.

SUMMARY

The literature in regard to the effect of age and the female sex hormones on the response of the host to disease agents is reviewed. It is shown that this literature may be divided into 3 groups of papers: first, those on the effect on antibody formation; second, on morphological change; and third, on the dispersion and concentration of disease agents in the skin.

It is shown that age increases the ability of the body to produce antibodies, that certain of the sex hormones can change the tissues so that resistance to infection is changed, that the dispersion or concentration of disease agents is affected by the endocrine pattern of the host, and that this concentration or dispersion affects the host's resistance to disease.

REFERENCES

1. Bullowa, J. G. M., and D. M. Kaplan: *Med. News, N. Y.* 83: 787. 1903.
2. Baumgartner, L.: *Yale J. Biol. & Med.* 6: 403. 1934.
3. Schultz, M. P.: *J. Exper. Med.* 60: 339. 1934.
4. Von Gröer, F., and K. Kassowitz: *Ztschr. f. Immunitätsforsch u. exper. Therap. Orig.* 28: 327. 1919.
5. Bay-Schmith, E.: *Klin. Wchnschr.* 8: 974. 1929.
6. Medical Research Council: Diphtheria, His Majesty's Stationery Office, London, 1923.
7. Jungeblut, C. W., and E. T. Engle: *J. A. M. A.* 99: 2091. 1932.
8. Aycock, W. L.: *Proc. Soc. Exper. Biol. & Med.* 34: 573. 1936.
9. Ratnoff, O. D.: *Proc. Soc. Exper. Biol. & Med.* 40: 248. 1939.
10. Huberman, J., and H. H. Israeloff: *J. A. M. A.* 103: 18. 1934.
11. Young, E. L., Jr., and G. A. Marks: *Surg., Gynec. & Obst.* 59: 610. 1934.
12. Ziskin, D. E.: *J. Dent. Research* 16: 367. 1937; Ziskin, D. E., S. N. Blackberg and C. A. Slanetz: *J. Dent. Research* 15: 407. 1936.
13. Sprunt, D. H., S. McDearman and J. Raper: *J. Exper. Med.* 67: 159. 1938.
Sprunt, D. H., and S. McDearman: *Endocrinology*, In Press; Sprunt, D. H., and S. McDearman: Given at Toronto meeting 1939 of the Am. Assoc. Immunol. Paper in preparation. Sprunt, D. H., and S. McDearman: Given at Toronto meeting 1939 of the Am. Soc. Exper. Path. Paper in preparation.
14. Duran-Reynals, F.: *J. Exper. Med.* 61: 617. 1935.
15. Parker, R. F: *J. Exper. Med.* 67: 725. 1938.

SEX DIFFERENCES IN ALBINO RATS TO TOXIC DOSES OF POWDERED RED SQUILL

D. GLEN CRABTREE, JUSTUS C. WARD AND JACK F. WELCH
From the Control Methods Research Laboratory of the U. S. Biological Survey
DENVER, COLORADO

THE INCREASING use of powdered red squill (*Urginea maritima*) as a specific raticide has led to considerable study concerning the nature of the rat-killing principle that has not as yet been isolated in pure form (6). In these investigations many workers observed that the female rat was more susceptible than the male to the powder or the extracts of red squill. Claremont (1) in 1922 reported that the female rat was about twice as susceptible to this toxic principle as was the male. Winton (2) in 1927 and LeBlanc (3) in 1938 confirmed the observations of Claremont.

The present work was undertaken to determine the relationship of the sex hormones to the difference in response of the female and the male rat to red squill powder.

EXPERIMENTAL

Albino rats were fasted for 24 hours, weighed and fed powdered red squill thoroughly mixed with dry bread crumbs and mineral oil in the proportion of 10% dry red squill powder, 5% mineral oil and 85% dry bread crumbs. The animals had access to water at all times and 24 hours after consuming the allotted doses they were given their regular diet. Death usually occurred overnight, but a few individuals succumbed as late as 2 or 3 days after consuming the toxic mixture.

The spayed females and the castrated males were allowed to recover from the operation approximately 30 days before being used as experimental animals. The castrate males that were to be injected with testosterone were also allowed to recover for a similar period before the daily injections of the hormone were begun.

The experiments were carried out in 4 groups corresponding to tables 1, 2, 3 and 4. All the animals in each group were fed the red squill mixture on the same day and were kept under exactly the same conditions. A uniform, previously standardized, powder prepared from a single red squill bulb was used throughout these investigations.

The animals that survived had completely recovered and were free from all symptoms of red squill poisoning within 5 days.

DISCUSSION

The first experiment (table 1) shows the difference in the susceptibility of normal female and male rats to the toxic principle of powdered red squill.

TABLE 1

No. and sex	Weight	Dose[1]	Result	No. and sex	Weight	Dose[1]	Result
	gm.				gm.		
1♂	178	200	Died	13♂	178	250	Died
2♂	181	200	Survived	14♂	182	250	Died
3♂	193	200	Died	15♂	191	250	Survived
4♂	198	200	Died	16♂	204	250	Died
5♂	201	200	Died	17♂	211	250	Died
6♂	206	200	Died	18♂	213	250	Died
7♂	207	200	Died	19♂	215	250	Died
8♂	210	200	Survived	20♂	216	250	Died
9♂	213	200	Survived	21♂	217	250	Died
10♂	215	200	Survived	22♂	219	250	Died
11♂	218	200	Died	23♂	225	250	Died
12♂	265	200	Survived	24♂	230	250	Survived
25♀	119	50	Survived	37♀	121	75	Survived
26♀	136	50	Survived	38♀	131	75	Died
27♀	139	50	Died	39♀	133	75	Died
28♀	141	50	Died	40♀	135	75	Died
29♀	144	50	Survived	41♀	136	75	Died
30♀	150	50	Died	42♀	137	75	Died
31♀	153	50	Survived	43♀	141	75	Died
32♀	154	50	Died	44♀	143	75	Died
33♀	155	50	Died	45♀	147	75	Died
34♀	156	50	Survived	46♀	148	75	Died
35♀	164	50	Survived	47♀	153	75	Died
36♀	175	50	Survived	48♀	167	75	Died

[1] Milligrams of red squill powder per kilogram of body weight.

TABLE 2

No. and sex	Weight	Dose[1]	Result	No. and sex	Weight	Dose[1]	Result
49♂	115	300	Survived	59 Castrate ♂	155	300	Died
50♂	124	250	Died	60 Castrate ♂	180	250	Died
51♂	125	200	Survived	61 Castrate ♂	191	200	Died
52♂	127	150	Survived	62 Castrate ♂	198	150	Died
53♂	130	150	Survived	63 Castrate ♂	199	150	Died
54♂	132	125	Survived	64 Castrate ♂	205	125	Died
55♂	182	125	Survived	65 Castrate ♂	207	125	Died
56♂	184	100	Survived	66 Castrate ♂	219	100	Died
57♂	193	75	Survived	67 Castrate ♂	230	75	Survived
58♂	220	50	Survived	68 Castrate ♂	238	50	Survived
69♀	125	300	Died	79 Spayed ♀	139	300	Died
70♀	139	250	Died	80 Spayed ♀	140	250	Died
71♀	148	200	Died	81 Spayed ♀	142	200	Died
72♀	156	150	Died	82 Spayed ♀	146	150	Died
73♀	158	150	Died	83 Spayed ♀	152	150	Died
74♀	159	125	Died	84 Spayed ♀	160	125	Died
75♀	164	125	Died	85 Spayed ♀	167	125	Died
76♀	170	100	Died	86 Spayed ♀	168	100	Died
77♀	176	75	Died	87 Spayed ♀	171	75	Died
78♀	183	50	Died	88 Spayed ♀	188	50	Survived

[1] Milligrams of red squill powder per kilogram of body weight.

One normal female survived a dose of 75 mg. per kilogram of body weight (mg./kg.) whereas 5 normal males survived a dosage of 200 mg./kg. of the powder. In the second and third series (tables 2, 3) the normal males survived a dose of 200 mg./kg. of red squill, but the castrate males died at 100 mg./kg.

TABLE 3

No. and sex	Weight	Dose[1]	Result	No. and sex	Weight	Dose[1]	Result
	gm.				gm.		
89♂	148	1000	Died	99 Castrate ♂	130	350	Died
90♂	149	750	Died	100 Castrate ♂	133	250	Died
91♂	155	500	Died	101 Castrate ♂	144	250	Died
92♂	169	400	Died	102 Castrate ♂	147	200	Died
93♂	179	400	Died	103 Castrate ♂	161	200	Died
94♂	185	400	Died	104 Castrate ♂	169	150	Died
95♂	188	400	Died	105 Castrate ♂	169	150	Died
96♂	195	250	Died	106 Castrate ♂	177	125	Died
97♂	205	200	Survived	107 Castrate ♂	178	125	Died
98♂	207	150	Survived	108 Castrate ♂	179	100	Survived
				109 Castrate ♂	195	75	Survived
				110 Castrate ♂	215	50	Survived
111♀	117	300	Died	123 Spayed ♀	124	300	Died
112♀	127	250	Died	124 Spayed ♀	132	250	Died
113♀	128	200	Died	125 Spayed ♀	137	200	Died
114♀	130	150	Died	126 Spayed ♀	143	150	Died
115♀	132	150	Died	127 Spayed ♀	144	150	Died
116♀	132	125	Died	128 Spayed ♀	145	125	Died
117♀	135	125	Died	129 Spayed ♀	146	125	Died
118♀	138	100	Died	130 Spayed ♀	146	100	Died
119♀	139	100	Died	131 Spayed ♀	153	100	Died
120♀	141	75	Died	132 Spayed ♀	155	75	Died
121♀	144	50	Died	133 Spayed ♀	161	50	Survived
122♀	146	30	Survived	134 Spayed ♀	182	30	Survived

[1] Milligrams of red squill powder per kilogram of body weight.

TABLE 4

No. and sex	Weight	Dose[1]	Result	No. and sex	Weight	Dose[1]	Result
	gm.				gm.		
135♂	145	400	Died	145♀	121	400	Died
136♂	162	350	Died	146♀	128	350	Died
137♂	172	300	Died	147♀	132	300	Died
138♂	187	250	Died	148♀	134	250	Died
139♂	190	200	Survived	149♀	139	200	Died
140♂	199	150	Survived	150♀	151	150	Died
141♂	211	100	Survived	151♀	152	100	Died
142♂	224	75	Survived	152♀	156	75	Died
143♂	227	50	Survived	153♀	166	50	Survived
144♂	245	25	Survived	154♀	171	25	Survived
155 Castrate ♂	165	400	Died	165[2] Inj'd castrate ♂	148	400	Died
156 Castrate ♂	173	350	Died	166 Inj'd castrate ♂	178	350	Died
157 Castrate ♂	179	300	Died	167 Inj'd castrate ♂	184	300	Survived
158 Castrate ♂	180	250	Died	168 Inj'd castrate ♂	197	250	Died
159 Castrate ♂	188	200	Died	169 Inj'd castrate ♂	222	200	Survived
160 Castrate ♂	195	150	Died	170 Inj'd castrate ♂	227	150	Survived
161 Castrate ♂	198	100	Died	171 Inj'd castrate ♂	238	100	Survived
162 Castrate ♂	203	75	Survived	172 Inj'd castrate ♂	239	75	Survived
163 Castrate ♂	217	50	Survived	173 Inj'd castrate ♂	242	50	Survived
164 Castrate ♂	221	25	Survived	174 Inj'd castrate ♂	250	25	Survived

[1] Milligrams of red squill powder per kilogram of body weight.

[2] Received 150 micrograms of testosterone propionate subcutaneously daily for 20 days preceding the experiment.

(table 2) and 125 mg./kg. (table 3) or above. The spayed females showed approximately the same susceptibility to the red squill as did the normal

females and the castrate males. The results of the fourth experiment (table 4) confirm those of the preceding ones and further demonstrate that when the hormone of the testes (testosterone) is supplied to castrate males their resistance to red squill is increased to the level shown by the normal males.

These data are similar to the findings of Holck and Kanan, as cited by Koch (4), who reported that the female albino rat was more sensitive than the male to such barbiturates as evipal, pernoston and amytal, but not to barbital. Lee (5), however, stated in 1935 that female mice and female rabbits were more resistant to nicotine poisoning than were the males of these species.

SUMMARY

The normal male rat is at least twice as resistant as the normal female to the toxic principle of red squill.

The presence or absence of the ovary has no effect on the susceptibility of the female to the toxic principle of red squill.

The presence of the testes or of the hormone of the testes (testosterone) seems to be the determining factor in the increased resistance of the male rat to powdered red squill.

REFERENCES

1. CLAREMONT, C. L.: *Analyst* 47: 60. 1922.
2. WINTON, F. R.: *J. Pharmacol. & Exper. Therap.* 31: 123. 1927.
3. LEBLANC, F. J.: A Thesis Submitted to the Faculty of Purdue University in partial fulfillment for the Degree of Doctor of Philosophy, June, 1938.
4. KOCH, F. C.: *Physiol. Rev.* 17: 153. 1937.
5. LEE, Y. E.: *J. Severance Un. Med. Col.* 2: 80. 1935.
6. O'CONNOR, M. G., R. E. BUCK AND C. R. FELLERS: *Ind. & Eng. Chem.* 27: 1377. 1935.

THE EFFECTS OF PROTEOLYTIC ENZYMES ON PURIFIED GONADOTROPIC HORMONES*

A. A. ABRAMOWITZ AND FREDERICK L. HISAW
From the Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

THE ACTION of pepsin and trypsin on pituitary gonadotropic hormones has been studied on several occasions, but in the hands of various investigators tryptic digestion of pituitary extracts has yielded divergent results. Bates, Riddle and Lahr (1) and Fevold (2) reported complete inactivation of pituitary FSH when treated with a commercial preparation of trypsin, while McShan and Meyer (3), Chen and van Dyke (4) and Greep (5) found that LH but not FSH was destroyed by this enzyme. Fevold (2) had previously reported the inactivation of LH by means of trypsin. McShan and Meyer employed both commercial and crystalline trypsin, while the remaining authors used various commercial tryptic preparations. Recently, Chow, Greep and van Dyke (6) found that crystalline trypsin was more effective than commercial trypsin in destroying the activity of an anterior pituitary extract containing the gonadotropic hormones.

The differential lability of FSH and LH to tryptic action (3, 4, 5) would afford additional chemical information concerning the separate identities of the two hormones, and, under certain conditions could be useful in the preparation of FSH completely free of LH. We, therefore, reinvestigated this problem with the object of studying the effects of several additional proteinases on FSH and LH, keeping in mind the possibility of employing enzymatic digestion as a means of purifying these extracts.

MATERIALS AND METHODS

The gonadotropic hormones used in this study were purified sheep FSH and LH and PU.[1] Crystalline trypsin, crystalline chromtrypsin,[2] commercial papain (Eimer and Amend) and clear saliva were used as proteolytic and amylolytic agents. The general procedure was to incubate at 37° C. a given amount of hormone and enzyme in a buffered solution at a proper PH,[3] and at various intervals to remove an aliquot sample for the assay of the hormone concerned. Digestion was stopped immediately by the precipitation of both hormone and enzyme as insoluble tannates.

* Supported in part by a grant from the Rockefeller Foundation.

[1] Kindly furnished by E. R. Squibb & Sons Co.

[2] These crystalline enzymes were prepared by Kunitz, M., and J. H. Northrup, *J. Gen. Physiol.* 18: 459, 1934, and Northrup, J. H., and M. Kunitz, *J. Gen. Physiol.* 16: 267, 1932.

[3] Since high alkalinity destroys both FSH and LH the PH at which experiments were performed was as close to neutrality as possible and yet within the effective range of the enzyme concerned.

Effects of enzymes on FSH

A purified extract containing 60 gm. equivalents pituitary tissue produced ovaries weighing between 40 to 45 mg. when a total of 3 gm. equivalents was tannated, emulsified in water and injected subcutaneously for 3 days, twice daily into 21-day-old rats. Thirty gram equivalents of FSH was added to 5.5 cc. Sorenson's phosphate buffer mixture at a proper pH for the enzyme concerned, and a small amount of the enzyme added. Controls contained the same amount of hormone but without enzyme. The reaction mixtures were placed in an incubator at 37° C., and at various intervals (3, 6, 11 hours) 10 gm. equivalents of the mixture was withdrawn, tannated, emulsified in water and injected. Each animal received a total dose of 3.0 gm. equivalents,

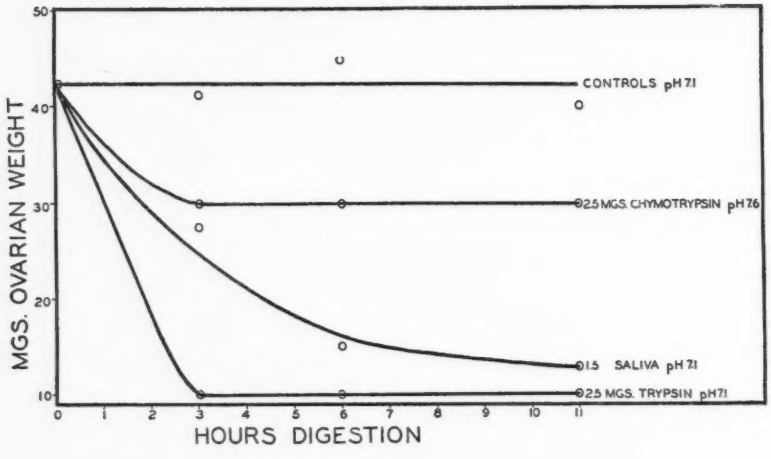


Fig. 1

3 to 5 animals being used for the assay of each sample of the digest. The results are shown in figure 1, a total of 40 rats being used.

Repetition of this experiment using graded amounts of chymotrypsin and trypsin gave results consistent with those shown above. A total of 100 rats injected with aliquot portions of a digest at 2, 4 and 7 hours indicated that 5 mg. of chymotrypsin was slightly more effective than 2.5 mg., but that there was little difference among 2.5, 1, and 0.5 mg. Digestion of FSH with 5 mg. chymotrypsin if allowed to proceed for 24 hours does not inactivate FSH completely. Trypsin, on the other hand, is decidedly more destructive. A total of 80 rats injected with aliquot portions of a digest after 2, 4 and 6 hours digestion showed complete inactivation with 1.0, 0.5 and 0.25 mg. trypsin, but little inactivation with 0.05 mg. Twenty rats injected with aliquot amounts of a papain digest (10, and 1 mg. papain) after 2, 4 and 6 hours digestion at pH 7.1 showed a partial inactivation of FSH, somewhat similar to that of chymotrypsin. The time curves for papain are not shown in figure 1 since a different preparation of FSH was used.

In figure 1, an aliquot portion was withdrawn from the control mixture immediately after solution, but not from any of the hormone-enzyme solutions. In the other experiments wherein graded amounts of enzyme were used, a sample was withdrawn in all instances as soon as the solution was prepared. The sample was tannated and injected in the usual manner. It was always found that FSH mixed with papain, trypsin and chymotrypsin

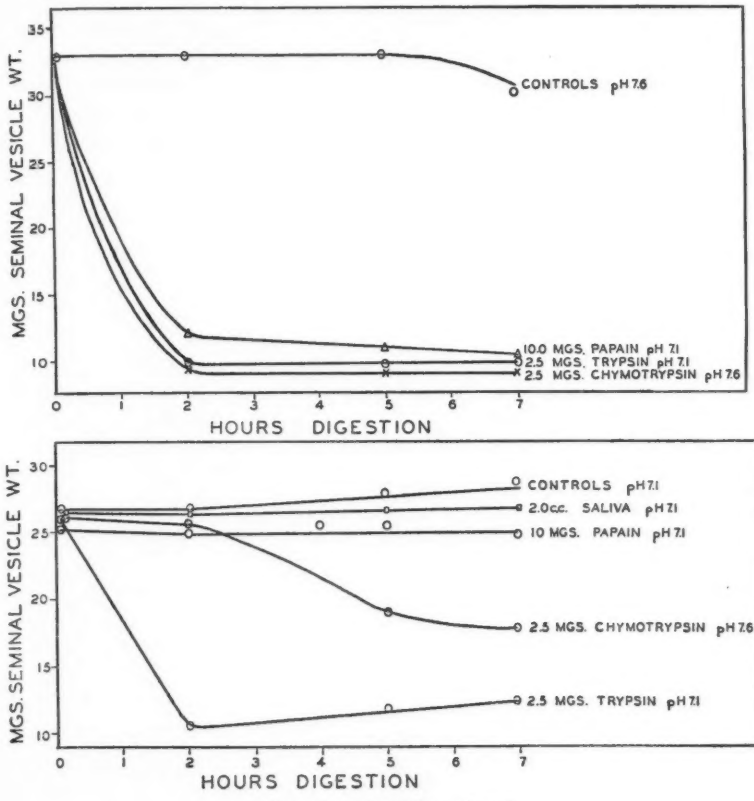

MGS. SEMINAL VESICLE WT.
CONTROLS pH 7.6
10.0 MGS. PAPAIN pH 7.1
2.5 MGS. TRYPSIN pH 7.1
2.5 MGS. CHYMOTRYPSIN pH 7.6
HOURS DIGESTION
MGS. SEMINAL VESICLE WT.
CONTROLS pH 7.1
2.0cc. SALIVA pH 7.1
10 MGS. PAPAIN pH 7.1
2.5 MGS. CHYMOTRYPSIN pH 7.6
2.5 MGS. TRYPSIN pH 7.1
HOURS DIGESTION

Fig. 2 (upper) and Fig. 3 (lower).

gave lower readings at 0 hours digestion than an equal amount of hormone alone. Addition of these enzymes to the same amount of tannated FSH resulted in no decrease of activity; in fact the FSH was slightly augmented.

Effects of enzymes on LH

A purified preparation of LH containing 50 gm. equivalents of pituitary tissue per cc. of extract was used. The LH was assayed by injecting it intraperitoneally into 21-day-old male rats for 4 days, twice daily, and weighing the seminal vesicles (including the coagulation gland) on the 5th day. Four

gram equivalents of LH injected in this manner, resulted in a seminal vesicle weight of around 26 mg. A total of 100 gm. equivalents was incubated with a given amount of enzyme at 37° C., in 10 cc. of Sorenson's phosphate buffer at a proper pH. Two cc. samples were withdrawn at 0, 2, 5 and 7 hours of digestion. The samples were tannated, emulsified in water so that each animal received a total dose of 4 gm. equivalents. The results are shown in figure 3, 120 animals being used.

Effects of enzymes on PU

Both male and female rats were used in assaying the effects of the enzymes on pregnancy urine hormone. Fifty rat units (Squibb) were incubated with the proteolytic enzymes in 10 cc. of Sorenson's buffer at a proper pH, and 3 cc. samples withdrawn at various intervals, tannated, emulsified in water

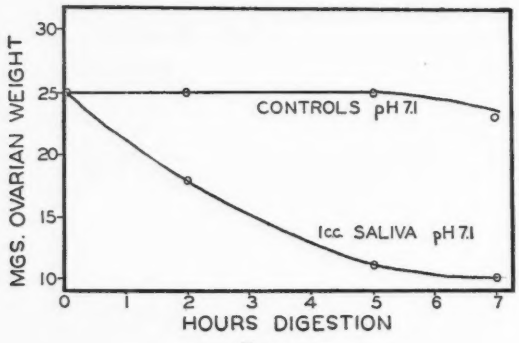


Fig. 4

and injected subcutaneously into male rats. Each animal received a total dose of 3 R.U. The injections were made twice daily for 4 days, and the animals killed on the 5th day. The results are shown in figure 2, a total of 64 rats being used. In the case of saliva, 50 R.U. was incubated with 1 cc. clear saliva in 10 cc. of buffer at pH 7.0. Three cc. samples were withdrawn from the digest and treated in the same fashion described above with the exception that the tannated samples were injected into female rats in a total dose of 4 R.U. per animal. The results of this experiment are given in figure 4, 18 animals being employed.

DISCUSSION

We have not confirmed the existence of a differential lability of FSH and LH to tryptic hydrolysis, as reported by McShan and Meyer (3), Chen and van Dyke (4), and Greep (5). From our curves it is evident that crystalline trypsin destroys FSH to the same degree and with much the same rate as it inactivates LH. We have, in agreement with McShan and Meyer, found a differential stability of these hormones to saliva, but the significance of this differential stability would be clearer if such experiments could be repeated with a pure ptyalin preparation. The stability of LH to salivary

digestion indicates that inactivation of FSH and PU by this enzyme was probably not due to bacterial influences, since LH has been found to undergo no destruction even after 11 hours of salivary digestion. Most of the enzymes under the conditions used in this study inactivate the gonadotropic hormones to a greater or lesser degree, which obviates their practical usefulness as agents for the digestion of the inert proteins present in these extracts. However, papain may prove to be useful in the purification of LH if the resulting hydrolysis of the inert proteins is sufficient to make such treatment practical.

SUMMARY

Crystalline trypsin at pH 7.1 completely destroys FSH, LH and PU. Crystalline chymotrypsin at pH 7.6 destroys PU completely, but only partly inactivates FSH and LH. Crude ptyalin at pH 7.1 destroys FSH and PU slowly, but does not inactivate LH. Commercial papain at pH 7.1 destroys PU completely, and FSH partly, but does not affect LH.

REFERENCES

1. Bates, R. W., O. Riddle and E. L. Lahr: *Proc. Soc. Exper. Biol. & Med.* 31: 1223. 1934.
2. Fevold, H. L.: *Cold Spring Harbor Symp. Quart. Biol.* 5: 93. 1937.
3. McShan, W. A., and R. K. Meyer: *J. Biol. Chem.* 126: 361. 1938.
4. Chen, G., and H. B. van Dyke: *Proc. Soc. Exper. Biol. & Med.* 40: 172. 1939.
5. Greep, R. O.: *Anat. Rec. Supp.* 2. 73: 23. 1938.
6. Chow, B. F., R. O. Greep and H. B. van Dyke: *Proc. Am. Physiol. Soc.* page 46. 1939.

CASE REPORT

SUPPRESSION OF LACTATION IN ACROMEGALY DURING ESTROGENIC THERAPY

D. J. STEPHENS

From the Department of Medicine of the University of Rochester School of Medicine and Dentistry and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals

ROCHESTER, NEW YORK

RECENT STUDIES have demonstrated the importance of the hypophysis in the complex hormonal factors which are responsible for lactation and have provided a rational explanation for the persistent galactorrhea occasionally observed in acromegaly and in other conditions not associated with the puerperium. Atkinson (1) reviewed the symptomatology of a large series of patients with acromegaly, including 607 females. He found references to 5 patients in whom galactorrhea had been described as part of the syndrome. In a series of 100 patients reviewed by Davidoff (2), persistent lactation occurred in 4 of 58 female patients. This consisted in the presence of small quantities of yellowish fluid resembling colostrum, evident on compression of one breast in 3 patients and of both breasts in the fourth. Davidoff refers to 3 other patients, including one male, in whom lactation had occurred.

The following patient is considered of interest as an instance of persistent lactation occurring in acromegaly, in which pituitary activity was apparently suppressed during estrogenic therapy.

CASE REPORT

A 48-year-old woman was admitted to the Out Patient Department of the Strong Memorial Hospital in May, 1938 with complaint of headache and enlargement of the hands. During the past 9 years she had noted gradual increase in the size of her fingers; 4 years before it had been necessary to have her wedding ring removed. The menopause had occurred at the age of 45; since then the hands and feet had increased rapidly in size. The shoe size had increased from 6½B to 9E and she was no longer able to find gloves large enough for her hands. The lower jaw had become prominent and the features had become coarse. There had been numbness, stiffness and aching of the fingers, of sufficient severity to cause insomnia. During the past 3 years there had been intermittent throbbing headache and pain in the occipital and posterior cervical regions. She had complained of thickness of the tongue and had noted that the voice

[1] The Schering Corporation, Bloomfield, N. J., kindly provided the progynon-B used in this study.

had assumed a lower pitch. During the past year a goiter had appeared. For a year or more the breasts had been enlarged, full and tense and she had been able to express milky secretion from the nipples; at times this stained her underclothing. In 8 years she had gained in weight from 150 to 193 pounds.

Her general health had been good in the past. There had been no visual symptoms. At times there had been questionable polydyspia and polyuria. The menstrual periods had always been regular until the abrupt onset of the menopause at the age of 45. She had had three uncomplicated pregnancies. The family history was unimportant.

The physical examination showed the characteristic physical abnormalities of acromegaly. There was prognathism and coarsening of the facial features. The hands were markedly enlarged and spadelike, with wide, massive fingers. Similar changes were present in the feet. The eyes showed no abnormalities. The visual fields were full. The tongue was large and thick. There was nodular enlargement of the thyroid. The heart was somewhat enlarged, with a soft systolic apical murmur. The blood pressure was 158 systolic, 105 diastolic. The breasts were moderate in size and were full and tense. It was possible to express from each nipple several drops of secretion having the macroscopic and microscopic characteristics of milk. Findings of the pelvic examination were negative except for some relaxation of the pelvic floor. Findings of the neurological examination were negative.

Laboratory findings were as follows. Red blood cells numbered 4,100,000 per cu. mm.; white blood cells, 5,500 per cu. mm.; hemoglobin, 11.8 gm. per 100 cc. Urinalysis showed no abnormalities. The blood Wassermann reaction was negative. The B.M.R. was +28%. The fasting blood sugar was 90 mg. %; ½ hour after 50 gm. of glucose by mouth, 158; 1 hour, 125; 2 hours, 98; 3 hours, 77; 4 hours, 73; 5 hours, 83. X-rays of the skull showed marked enlargement of the sella turcica.

Intramuscular injections of estradiol monobenzoate in oil[1] were begun on June 28, 1938. After 5 weekly injections of 2000 R.U. each, there was no change in the symptoms. The dose was increased to 10,000 R.U., once a week. After the fifth injection of the larger dose, there was marked symptomatic improvement. The tense, 'frozen' feeling of the skin and subcutaneous tissues had disappeared. The patient said that she felt as though she had been 'thawed out.' The numbness, stiffness and aching of the hands had disappeared, as had the insomnia and the headache. Lactation had definitely decreased; only a small amount of material resembling colostrum could be expressed from the breasts. After the fifteenth injection of 10,000 R.U., secretion could no longer be expressed from the nipples. The patient had noted that the breasts had become smaller and the tense feeling had disappeared. She was enthusiastic about the general symptomatic improvement and insisted that she felt well for the first time in several years. The blood pressure was 120 systolic, 80 diastolic. Otherwise there was no definite change in the physical findings or in her general appearance. The B.M.R. was +27%. The fasting blood sugar was 80 mg. %; ½ hour after 50 gm. of glucose by mouth, 120; 1 hour, 83; 2 hours, 100; 3 hours, 74; 4 hours, 74; 5 hours, 85. On December 6th, 1938 the injections were discontinued; the patient had been given a total of 190,000 R.U. of estradiol monobenzoate in oil over a period of 23 weeks. Eight days after the last injection, vaginal bleeding began and continued for 10 days. Within 2 months after the injections were discontinued, she was again lactating and stiffness of the hands had reappeared.

DISCUSSION

Experimental studies during the past ten years have established the conception that lactation is induced by a positive hormonal stimulus from

the anterior pituitary gland. It is generally agreed that the ovarian hormones are responsible for the growth and development of the breast and that lactation is dependent on a delicate hormonal balance involving the anteriory pituitary, ovaries, adrenals and perhaps the thyroid (3). A modern concept of the factors responsible for physiological lactation has been presented by Nelson (4), and may be summarized as follows. The high concentration of ovarian hormones during pregnancy is conducive to glandular development but inhibitory to lactation. This restraining influence may operate either by suppressing secretion (or release) of the lactogenic hormone from the anterior pituitary or by a direct inhibitory effect on the mammary glands. With the decline in the level of estrogenic substances at parturition, the inhibiting influences are removed, lactogenic hormone is secreted and lactation occurs.

Injections of anterior lobe extracts have been shown to stimulate lactation in adequately developed mammary glands in numerous species (5). Riddle, Bates and Dykeshorn (6) have applied the term 'prolactin' to the hypophyseal principle which acts as the affective stimulus to milk secretion in the prepared mammary gland and have shown that this principle is apparently distinct from growth and gonad stimulating effects. Prolactin has been used for the stimulation of breast milk secretion by nursing mothers (7).

There is clinical and experimental evidence that pituitary activity, including the lactogenic function, may be inhibited by the administration of the sex hormones. The literature contains several references to the treatment of acromegaly (8) and of pituitary basophilism (9) with estrogenic hormones, given for the purpose of suppressing anterior pituitary activity. Zondek (10) and others (11) have presented convincing evidence which indicates that anterior pituitary functions, in particular the gonadotropic and growth principles, may be suppressed by the administration of large doses of estrogenic substance. In the experimental animal, lactation may be suppressed or prevented by the administration of testosterone propionate (12) or estrogen (13). Several investigators have shown that lactation during the puerperium may be promptly and effectively inhibited by the administration of the sex hormones, presumably due to suppression of anterior pituitary function (14). The observations of Zondek (10) and of Reese (15) suggest that the inhibitory effect of the sex hormones may be due to interference with delivery of the hypophyseal principles, rather then to inhibition of production of the various hormones by the anterior lobe cells.

Nelson (3) has shown that in the experimental animal previously treated with estrone, the lactation induced by the injection of pituitary extracts failed to occur if the estrogenic substance was continued during the administration of pituitary extracts. While the weight of experimental evidence seems to indicate that the inhibitory effect of the sex hormones on lactation is due to suppression of pituitary activity, it has been suggested that the estrogens act specifically on the mammary gland, in some way preventing the action of the lactogenic hormone of the anterior pituitary (8c). Either

or both of these effects may have been in operation in the patient described in this report; the striking, general symptomatic improvement may, perhaps, be interpreted as additional evidence of inhibition of pituitary activity.

SUMMARY

A patient with acromegaly is described, in whom persistent secretion from both breasts, macroscopically and microscopically characteristic of milk, had become established. During estrogenic therapy, there was suppression of lactation, associated with striking subjective clinical improvement, lowering of an elevated blood pressure, and slight increase in glucose tolerance. There was no change in the general clinical appearance or in the basal metabolic rate during the administration of estrogenic substance.

REFERENCES

1. Atkinson, F. R. B.: Acromegaly, John Bale, Sons & Danielson, Ltd. London, 1938.
2. Davidoff, L. M.: *Endocrinology* 10: 461. 1926.
3. Nelson, W. O.: *Am. J. Anat.* 60: 341. 1937.
4. Nelson, W. O.: *Physiol. Rev.* 16: 488. 1936.
5. Folley, S. J.: *Lancet* 2: 389. 1938.
6. Riddle, O., R. W. Bates, and S. W. Dykeshorn: *Am. J. Physiol.* 105: 191. 1933.
7. (a) Kurzrok, R., R. W. Bates, O. Riddle and E. J. Miller: *Endocrinology* 18: 18. 1934.
   (b) Ross, J. R.: *Endocrinology* 22: 429. 1938.
8. (a) Kirklin, O. L., and R. M. Wilder: *Proc. Soc. Staff. Meet. Mayo Clin.* 11: 121. 1936.
   (b) Shire, I., and E. P. Sharpey-Schafer: *Clin. Sci.* 3: 413. 1938.
   (c) Foss, G. L., and P. Phillips: *Brit. M. J.* 2: 887. 1938.
9. (a) Dunn, C. W.: *Lancet* 2: 344. 1937.
   (b) Gill, A. M.: *Lancet* 2: 72. 1937.
   (c) Pardee, I.: *Proc. Assoc. Res. Nerv. & Ment. Dis.* 17: 609. 1938.
10. Zondek, B.: *Lancet* 2:842. 1936.
11. Allen, E.: Sex and Internal Secretions. The Williams and Wilkins Co., Baltimore, 1939.
12. (a) Robson, J. M.: *Proc. Soc. Exper. Biol. & Med.* 36: 153. 1937.
   (b) Folley, S. J., and S. K. Kon: *Proc. Roy. Soc., London, s.B.*, 124: 476. 1938.
13. (a) DeJongh, S. E.: *Acta brev. Neerland.* 3: 52. 1933.
   (b) Robson, J. M.: *Quart. J. Exper. Physiol.* 24: 337. 1935.
   (c) Nelson, W. O.: *Am. J. Anat.* 60: 341. 1937.
14. (a) Brea, A. M., and C. C. Sontag Gandra: *Semana med.* 44: 1463. 1937.
   (b) Lindeman, W.: *Zentralbl. f. Gynäk.* 62: 159. 1938.
   (c) Kurzrok, R., and C. P. O'Connell: *Endocrinology* 23: 476. 1938.
   (d) Foss, G. L., and P. Phillips: *Brit. M. J.* 2: 887. 1938.
15. Reese, R. P.: *Proc. Soc. Exper. Biol. & Med.* 39: 77. 1938.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Authur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

BERK, J. E. Clinical experience with dihydrotachysterol in the management of idiopathic hypocalcemia. Report of a case. *To appear in this Journal.*

A case of hypocalcemia is reported. The etiology is not certain but appeared to be of hypoparathyroid origin. The more common therapeutic agents were ineffectual, but dihydrotachysterol (A.T. 10) proved very successful. Detailed studies are presented of the serum calcium levels during the period of hospitalization and follow-up.

CANZANELLI, A., R. GUILD AND D. RAPPORT. Further observations on the effect of thyroglobulin and O_2 consumption of tissues in vitro. *To appear in this Journal.*

Using a Warburg-Barcroft differential manometer system the effect of thyroglobulin and thyroxin upon the in vitro O_2 consumption of guinea-pig kidney, testis and heart was investigated. At lower levels of metabolism thyroglobulin stimulated the metabolism of each of these tissues. However, there appeared to be a 'ceiling' above which thyroglobulin could no further elevate the O_2 uptake, and this 'ceiling' was higher, in the case of kidney and heart, in serum than in a salt buffer mixture. Thyroxin generally had no stimulating effect in either the salt buffer mixture or the serum. In two experiments in serum, however, a stimulating effect appeared, raising the possibility that thyroxin under certain circumstances may be made physiologically active by contact with serum.

CLEGHORN, R. A., C. W. J. ARMSTRONG AND D. C. AUSTEN. Clinical and chemical observations on adrenalectomized dogs maintained by a diet high in sodium salts and low in potassium. *To appear in this Journal.*

Adrenalectomized dogs have been maintained for periods up to 4½ months on diets high in Na salts and low in K similar to that employed by Nilson (1937). The not infrequent occurrence of spontaneous crisis was characterized by refusal of food, vomiting, anuria and loose, strawberry-coloured stools, and sometimes frank melaena. Auscultation of the chest revealed moist râles and rhonchi. The heart rate was frequently accelerated but at other times the extreme bradycardia, so often encountered in adrenal insufficiency in dogs on a diet containing little Na and an average amount of K, was found. Blood chemical values showed the electrolyte deviation characteristic of insufficiency and decrease in plasma volume. Autopsy of such animals revealed edema of the lungs and, in addition to the upper gastrointestinal tract congestion and ulceration, extraordinarily intense congestion and hemorrhagic areas in the large bowel quite in excess of anything we have ever observed after death from adrenal insufficiency of dogs receiving food of unadjusted Na and K content. The spontaneous crisis occurring in the salt-maintained dog corresponds closely to that precipitated by feeding large amounts of K to these animals, though severe hypoglycemia has also been encountered. In many instances we found it possible to restore the dogs from even severe spontaneous crises by intravenous injections of hypertonic saline and glucose, without resort to the use of adrenal cortical extract. Later the use of normal saline and forced feeding until an adequate intake of food and salts, voluntarily taken, assured their maintenance.

HAIR, G. W., AND J. F. MEZEN. A study of the functional innervation of the hypophysis. *To appear in this Journal.*

The functional relation between the parasympathetic component of the facial nerve and the hypophysis has been investigated by electrical stimulation of the nerve at the geniculate ganglion and bilateral destruction of the facial nerve. Stimulation of the facial nerve at the geniculate ganglion did not produce ovulation in adult rabbits. Bilateral destruction of the facial nerve did not prevent the normal ovulatory response to copulation.

HAMILTON, J. B., AND W. R. C. GOLDEN. Responses of the female to male hormone substances. With notes on the behavior of hens and newly-hatched female chicks. *To appear in this Journal.*

Administration of crystalline male hormone substances can produce definite cock-like behavior in either newly-hatched female chicks or in the mature hen. The degree of crowing behavior was not proportional to the degree of comb growth, instead seeming to be largely dependent upon factors in the individual bird in addition to the degree of androgenic stimulation. Potentially masculine behavior patterns appear to be preformed but latent in the female until activated by adequate stimulation by androgenic substances. They can be exhibited by the androgen-treated female even though no cock is present to serve as an example of such behavior. The type of masculine behavior shown by the female receiving male hormone substance tends toward the type of behavior shown by the male of the species. The limited data presented in this report would suggest, however, that at least under the experimental conditions employed, the females responded to the androgen, testosterone propionate, to a lesser degree than the males. Discussion is given of the ability of the female to produce and to respond to male hormone substances.

HOUCHIN, O. B. The influence of anterior pituitary extract on the proteins of the liver. *To appear in this Journal.*

Extracts of the anterior lobe of the pituitary at levels from 1 to 20 mg. did not cause a true hypertrophy of the liver of guinea pigs. The percentage of water in the liver did not vary significantly. The alkali soluble protein of the liver was decreased by a principle in the AP, denoted by the term protein metabolism hormone. This decrease ranged from 35 to 53% of the normal value. An increase was noted in the other fractions of the liver studied. Indications from the data were that the optimal level was low. The interval of time before killing was shown to affect the response slightly. Two other fractions, lactogenic and thyrotropic, of the original preparation were shown to have only very slight activity. This protein metabolism hormone was further shown to be an entity by its being destroyed with .1 N NaOH at 60°C. for 90 minutes, while the fat metabolism hormone remained constant.

KUNSTADTER, R. H. The treatment of hypogenitalism in the male with the gonadotropic principle of pregnant mares' serum. *To appear in this Journal.*

The gonadotropic hormone derived from pregnant mares' serum was administered intramuscularly to 14 boys who exhibited hypogenitalism, with and without cryptorchidism. Intradermal tests to indicate individual sensitivity were performed on all patients before the preparation was injected intramuscularly. Local reactions following intramuscular administration occurred in 4 patients. Definite improvement, as evidenced by growth of the genitalia, the appearance of secondary sex characteristics, or descent of undescended testes occurred in 8 patients. Additional boys are being treated in order to determine an effective average dose of hormone for the treatment of such patients.

LERMAN, J., AND W. T. SALTER. The relief of myxedema with proteins of extrathyroidal origin. *To appear in this Journal.*

Iodinated serum protein and its acid-insoluble degradation products produced 13 successful responses in myxedema and cretinism and 2 successful responses in thyroidectomized rabbits. This material originated from the brief treatment of blood proteins with compound solution of iodine. In terms of iodine, the activity of iodoprotein was about 1/400th that of whole thyroid, and the activity of the acid-insoluble iodopeptone was about 1/200th. Like thyroglobulin, iodoprotein can be fractionated by the enzymes pepsin and trypsin or by alkaline hydrolysis into 'thyroxine-like' ('T') and into 'diiodotyrosine-like' ('D') fractions. The former possess all the activity present in the iodoprotein, whereas the latter are inert. The acid-insoluble fraction can further be split by drastic alkaline hydrolysis into material resembling iodothyronine derivatives. The activity of this material is of the order of crystalline diiodothyronine, namely 1/30th to 1/40th that of whole thyroid.

MARLOW, H. W. Properties of ovarian extracts. *To appear in this Journal.*

The injection of residual ovarian extracts and extracts of follicular fluid cause a hypertrophy of the uterus of young immature female rats. The assayed extracts giving this response show a decided break in the curve at about .4 rat unit. Solutions made from crystalline estrone and estradiol do not exhibit such

a break but increases the hypertrophy of the uterus in all the dosages given. It is thought then, that the residual ovarian extracts and the follicular fluid extracts contain the same estrogenic compound; that the response resembles estrone and not estradiol and that if the principal estrogenic compound is estradiol, some inhibiting substance is present to prevent the exhibition of the full effect of the hormone.

NATHANSON, I. T., AND L. E. TOWNE. The urinary excretion of estrogens, androgens and FSH following the administration of testosterone to human female castrates. *To appear in this Journal.*

Testosterone propionate was administered to 4 human female castrates who had severe vasomotor symptoms. All had symptomatic relief and revealed changes in the vaginal smear toward the cornified type. Assays of the 24-hour urine specimen showed a moderate to marked increase in the output of total estrogens, a slight rise in the androgens and a decrease in the FSH levels. There seemed to be a definite correlation between the laboratory findings and the clinical changes.

NEUFELD, A. H., AND J. B. COLLIP. The effect of pituitary extracts on ketonuria, fat content and fat distribution in the liver and tissues of mice. *To appear in this Journal.*

A study has been made of the ketogenic properties of various pituitary fractions. Points of special interest arising out of these experiments are: association of the ketogenic activity with protein fractions; thermostability of the ketogenic substance over a wide pH range which is confined to the acid side; presence of ketogenic substance in isofiltrates obtained after heating active protein fractions in an acid medium; presence of ketogenic activity in the precipitate thrown down with alcohol from such isofiltrates. In 24-hour fasted mice (dba) active ketogenic preparations bring about a fatty infiltration of the liver. Total body fat does not show an appreciable change. In 48-hour fasted animals the high liver fat is associated with a low total body fat. Preparations containing the specific metabolic stimulant produce an appreciable loss of total body fat. This is associated with a low liver fat content in 24-hour fasted animals. Preliminary experiments on the administration of ketogenic preparations together with the specific metabolic stimulant show an enhanced loss of total body fat in mice.

NEUFELD, A. H., AND J. B. COLLIP. Further studies on the antagonist to adrenalin hyperglycemia in pituitary extracts. *To appear in this Journal.*

Evidence has been presented to show that certain posterior pituitary preparations produce on administration to mice, rats, rabbits, monkeys and a dog, an antagonism to adrenalin hyperglycemia. A number of the properties of the substance responsible for the antagonism are given. This substance can be differentiated from the factor responsible for the immediate metabolic response. The evidence available does not indicate a relationship of this substance to other well known posterior pituitary principles.

SHAY, H., J. GERSHON-COHEN, K. E. PASCHKIS AND S. S. FELS. The effect of large doses of testosterone propionate (oreton) on the female tract of the very young rat. *To appear in this Journal.*

By administering large doses of testosterone propionate, beginning at the age of one day, ovarian tumors were produced in female rats. These tumors were shown to be lutein cysts. In some animals treated identically, atrophy of the ovaries without luteinization occurred. The size of the lutein cysts was not directly related to the total dose of testosterone propionate nor to the duration of treatment. The estrus cycle was not completely suppressed; even after high total doses of testosterone propionate occasional cycles appeared in some animals with lutein cysts and with macroscopically atrophic ovaries. In one of the macroscopically atrophic ovaries, mature follicles were found. The uterus presented progestational pictures in most cases, but in 3 animals estrus changes were found. Occasional maturation of follicles can occur even after large doses of testosterone propionate. Since cycles occurred in animals bearing lutein tumors, the question may be raised how long the lutein tissue remains functional. This question cannot be answered at the present moment. The present conception is that administration of sex hormones affects the gonads by depression of the pituitary. Under this theory we would have to assume release of luteinizing pituitary hormone from the otherwise depressed pituitary. However, a direct influence of testosterone on the female gonad has to be envisaged as another alternative explanation.

SLEETH, C. K., AND E. J. VAN LIERE. The size of the spleen and the adrenals during pregnancy and the puerperium. *To appear in this Journal.*

The spleen weight-body weight (SW/BW) and the adrenal weight-body weight (AW/BW) ratios were determined in 52 normal female guinea pigs. These ratios were also determined in 33 guinea pigs during the 7th week of gestation and in 29 guinea pigs approximately 72 hours after parturition. With the exception of the experimental conditions, all 3 groups of animals belonged to the same statistical population. In the pregnant animals the spleen was found to be about 15% smaller than in the control group. This finding agrees with Barcroft's work. There was, however, no significant difference in the weight of the spleens of the post-partum group and the control animals. The adrenals in the pregnant

animals were of normal size. This finding does not substantiate the prevailing opinion that the adrenals hypertrophy during pregnancy. In the post-partum group, however, the adrenals were found to be increased in size about 34%; the possibility of this occurring by chance was less than 1 in a 100. The rapid increase in weight of the adrenals in the post-partum period can best be explained as being due to a redistribution of fluid following the hypervolemia of pregnancy.

SPURR, C. L., AND C. D. KOCHAKIAN. The effect of androgens on the survival of adrenalectomized rats. *To appear in this Journal.*

The month-old male adrenalectomized rat has an average survival time of 8.7 days which is decreased to 7.1 days by injecting twice daily with 0.1 cc. of sesame oil. The administration of androgens (urinary androgenic extract 50, 150, 250 and 250 I.U.; androstenedione 5, 10, 30 and 30 I.U.; testosterone acetate 14 I.U. and testosterone propionate 50 and 125 I.U. per day) resulted in a marked decrease in survival time which was roughly proportional to their physiological activity. At 250 I.U. (urinary extract) the survival time was decreased to 2.7 days.

STERNHEIMER, R. The effect of a single injection of thyroxine on carbohydrates, protein and growth in the rat liver. *To appear in this Journal.*

The changes in the chemical composition of the rat liver, after a single injection of thyroxine, vary according to the time elapsed after the injection. The chief loss in liver glycogen occurs within 6 hours after the injection, i.e. during the 'latent period'; it is followed by an increase in glycogen above normal, beginning 48 hours after injection during a period in which the O_2 consumption of the animal is increasing. Liver sugar and blood sugar remain unchanged when the decrease in glycogen occurs, but rise simultaneously with the liver glycogen. The lactic acid content of the liver remains unchanged. The glycogen content of the entire animal shows variations similar to those of the liver glycogen. The protein content of the liver, determined simultaneously, rises markedly in the first period, to decrease later toward normal values in almost linear proportion to the increase in liver glycogen, suggesting gluconeogenesis from protein. Fresh and dry weight of the liver increase. The variations in carbohyrdates observed in the liver present a close analogy to changes described in growing tissues. Histological studies, performed at the same intervals, reveal morphological changes described in regenerating liver. An increased number of mitoses demonstrates that active growth of the liver cells takes place.

TALBOT, N. T., AND G. O. LANGSTROTH. A rapid extractor for urinary androgens. Factors to be considered in the preparation of extracts for colorimetric assay. *To appear in this Journal.*

A small and convenient apparatus which permits extraction of about 90% of urinary androgens in 1 hour with benzene is described. Recovery experiments made in part with the aid of this apparatus show that a significant amount of androsterone and of dyhydroiso-androsterone is lost in the preparation of urine extracts for colorimetric assay. The chief loss occurs when the ether extract is cleared with decolorizing charcoal. The facts presented lead to the conclusion that the amount of androgenic 'sterone' substances determined by the usual colorimetric methods which include treatment with decolorizing charcoal as part of the procedure are not an accurate index of the original 'sterone' content of the urine.

TEAGUE, R. S. Biological assay of the melanophore hormone of the pituitary gland. *To appear in this Journal.*

A method for the biological assay of the melanophore hormone of the pituitary is described, using the hypophysectomized frog as the test object and alkali-treated U.S.P. posterior pituitary powder as the reference standard. A unit of melanophore hormone is defined as the activity of 1γ of the alkali-treated standard, when injected into a 30 gm. hypophysectomized frog.

TEAGUE, R. S. The relation of the melanophore hormone of the pituitary gland to oxygen consumption of the rat. *To appear in this Journal.*

Preparations of the pituitary gland rich in melanophore hormone, obtained from various sources and prepared by different methods, vary considerably in their effect on O_2 consumption in rats. Hypophysectomized or thyroidectomized rats are subject to the same variations in response as normal animals. A pituitary extract of Collip and a melanophore hormone extract prepared from whale anterior lobe, although having a similar melanophore hormone content, showed marked differences in their action on O_2 consumption in rats, in that Collip's extract in large doses was capable of stimulating metabolism while the whale preparation was inactive. When the melanophore hormone in pitmelanin was destroyed by HCl or by tryptic digestion, the product produced even more consistent metabolic stimulation than the original extract. The evidence here warrants the conclusion that the melanophore hormone of the pituitary gland is not identical with a substance in pituitary extracts which would increase the B.M.R. This statement finds support in the very recent report of Denstedt and Collip that pituitary fractions have been obtained in which the melanophore hormone and the 'specific metabolic principle' apparently

do not parallel one another. The data are insufficient to demonstrate the presence of a specific metabolic principle of the hypophysis, since metabolic stimulation was produced with a pituitary extract after treatment with acid and after tryptic digestion, and since metabolic responses were occasionally seen with extracts of muscle, liver, and kidney.

TISLOWITZ, R. The colchicine test as a method for determining the time of onset and the duration of action of male active substances. *To appear in this Journal.*

Modification of the test of Loewe and Voss by the use of colchicine permits of a finer analysis of the effect of male hormones on the seminal vesicles than by the usual methods in which the enlargement of the whole seminal vesicle is observed. Observation of the action or a single injection of a male active substance throughout its course of action is made possible. On comparison of different active substances the more accurate histological analysis probably permits the establishment of differences in the mechanism of the action. The first profuse formation of mitoses in the seminal vesicles of young castrated mice is observed 24 to 25 hours after injection of 150γ of testosterone and 27 to 31 hours after injection of 180γ of testosterone propionate. Their activity is still maintained after 58 hours, while doses 10 times smaller develop much weaker activity after this time. In general, testosterone propionate shows the most numerous mitoses and the most uniform distribution. The addition of 'X' substance to testosterone increases the activity of the latter.

VOLLMER, E. P., A. S. GORDON, I. LEVENSTEIN AND H. A. CHARIPPER. The effects of hypophysectomy upon the blood picture of the rat. *To appear in this Journal.*

Erythrocyte counts and hemoglobin determinations in 23 hypophysectomized rats were found to fall from 30 to 40% below normal within 6 to 9 weeks after operation. No anemia was found in 11 operated control rats or in 5 starved normal control rats. The reticulopenia of hypophysectomized rats, described by other workers, was confirmed; but the reticulocyte counts in some controls, at various times, were also low. No distinct trend was found in the total or differential white cell counts of the hypophysectomized animals. The bone marrow of the hypophysectomized animals showed a distinct hypoplasia, with increased vacuolization and relatively more fat cells. The various possible causes of the post-hypophysectomy anemia are discussed.

WATSON, B. A. Clinical significance of glycosuria. *To appear in this Journal.*

For a 7-year period every student at the University of Minnesota, having glycosuria on routine examination was further studied by means of a glucose tolerance test. A complete history and physical examination was obtained with special reference to age, sex, family history of diabetes, previous glycosuria, weight variation, fatigue and symptoms of diabetes. Analysis of the data shows that in 51,096 urine examinations glycosuria was found in 1.14%, or 583 cases. Of the 583 patients, 90 or 15.44% had evidence of a disturbed carbohydrate metabolism as judged by the glucose tolerance test. Obesity in this age group (mean 22.7 years) did not influence the results of the glucose tolerance test as the mean blood sugar values of the underweight group were the highest and those of the overweight group the lowest. Seventeen cases of glycosuria had a family history of diabetes, were 20% or more overweight, and the mean blood sugar values of their glucose tolerance tests were within normal variations. One hundred and nine individuals had a family history of diabetes and their mean blood sugar values were also well within normal limits, raising the question of the importance of family history as a predisposing factor to the development of diabetes in this age group. The importance of a routine glucose tolerance test on patients with glycosuria is suggested.

GONADS

DIPPLE, A. L. The rôle of hysterectomy in the production of menopausal symptoms. *Am. J. Obst. & Gynec.* 37: 111. 1939.

A group of 42 women who had been sterilized by hysterectomy were questioned in person in regard to the various signs and symptoms of menopause. However, the cases were evaluated on the basis of hot flushes only. In order to obviate the age factor, no patient was included in the study who was over 35 at the time of operation; the average age being 29 years. The time interval between hysterectomy and interview varied between 1.6 and 8.0 years, the average interval being 4.1 years. The parity distribution was, primiparas 5, multiparas 37. Twenty-five of the patients were white and 17 colored. With 4 exceptions, the operations were carried out on patients who were, or had recently been, pregnant. In 6 cases one ovary was removed along with the uterus. Except for one panhysterectomy the operations were all supravaginal hysterectomies and were done by a number of operators. The results of this study showed that the incidence of menopausal symptoms following hysterectomy on the pregnant or recently pregnant patient, namely 16.6% was decidedly less than that previously reported when hysterectomy was done in the presence of fibromyomas or pelvic inflammatory disease, that is, 43.9 to 81.0%. It suggests that

hysterectomy with ovarian conservation brings about menopausal symptoms through injuring the ovarian blood supply rather than through the absence of the uterus per se.—Author's Abstract.

Dorfman, R. I., and J. B. Hamilton. Urinary excretion of androgenic substances after intramuscular and oral administration of testosterone propionate to humans. *J. Clin. Investigation* 18: 67. 1939.

To 2 men with testicular insufficiency androgenic material was given, 20 mg. in oil intramuscularly daily, or 60 mg. orally. The urinary loss of androgens was brought to levels higher per 24 hours than is characteristic of normal adults. Symptoms were relieved by the parenteral administration, not by oral method, even when the dose was 120 mg. daily. In the latter case urinary loss was far above normal levels. The number of doses is not specified, but the authors suggest that the maintenance of androgenic concentration in the blood was probably not constant in the case of oral therapy as in the use of intramuscular injections. The per cent excretion can not be calculated because the androgen in the urine is not identified. It appears to be a fairly large fraction of that injected, if calculated as androsterone. Intestinal absorption is obvious.—E. L. S.

Effkemann, G. Pseudomenstruation as a cause of sterility in mature women (Die Pseudomenstruation als Sterilitätsursache bei der erwachsenen Frau). *Zentrlbl. f. Gynäk.* 63: 153. 1939.

Following discussion of the possibility, significance, and mechanism of anovulatory bleeding, a summary report is presented on 81 sterile women, in 66 of whom progestational endometrium was found. In the other 15 the endometrium was of proliferative type although obtained just before or at the beginning of flow. Of these, 10 had cycles varying from 3 to 5 weeks, with flow which is recorded as excessive, whereas 5 had no anomalies of cycle length or amount of flow. It is not clear just how the author differentiates two types, one due to persistent follicle action, the other to cyclic follicle action with atresia rather than ovulation and luteinization. The occurrence of anovulatory flow is interpreted as evidence of hypofunction of the ovaries.—E. L. S.

Emmrich, J. P. Medical induction of labor (Die medikamentöse Einleitung der Geburt). *Zentrlbl. f. Gynäk.* 63: 199. 1939.

After citation of statistics about the hazard of prolonged gestation, and the results of inductions, the author presents two series: in the first labor was induced by castor oil and quinine in 47% of 455 trials, with the infrequent use of thymophysin. In thesecond (63 cases) success was had in 74.6% following the use of 3000 to 10,000 units of estrogenic substance intravenously, followed by posterior pituitary at half-hour intervals, 1 Vögtlin unit for each of not over 3 doses. There were no obvious difficulties, and those cases which did not respond at once were derelivered later. In some cases the failures came from the very small doses of estrin.—E. L. S.

Flaks, J., A. Ber and R. Pakszwer. Effects of tumor grafts on the action of folliculin in mice (Influence des tumeurs greffées sur l'action de la folliculine chez les souris). *Compt. rend. Soc. de Biol.* 128: 775. 1938.

Tumor grafts cause cessation of estrus in mice but whether through an action on the ovary or on the uterus is not known. Tumors were transplanted into 12 spayed mice and 8 of these were given injections of folliculin. Estrus reappeared in these. The inhibitory action of the tumor is, therefore, not on the uterus.—J. C. D.

Fluhman, C. F. Estrogenic hormones. Their clinical use. *California and West. Med.* 49: 362. 1939.

This is a discussion of the indications and contraindications for the use of estrogenic hormones. These substances should not be employed in sufferers from abnormal uterine hemorrhage. The fantastic claims made for the estrogenic hormones have caused them to be regarded by many physicians with suspicion. It is hoped that a sane consideration of their function in normal physiology may lead to a correct evaluation of their place as therapeutic agents.—I. B.

Frank, J. A. Some properties of sperm extracts and their relationship to the fertilization reaction in *Arbacia punctulata*. *Biol. Bull.* 76: 190. 1939.

Extracts of Arbacia sperm agglutinate eggs of the same species. This egg-agglutinating substance is found only in extracts of sperm and is not secreted into sea water. It is a colloidal substance which disintegrates upon standing. Mg and Ca ions are essential for its extraction. Arbacia eggs are not activated by immersion in sperm extracts.—E. L.

Friedgood, H. B., and M. A. Foster. The experimental production of ovulation, luteinization and cysts of the corpus luteum in adrenalectomized anestrous cats. *Am. J. Physiol.* 123: 237. 1938.

The ovarian follicles of 13 normal anestrous cats and 11 adrenalectomized anestrous cats were matured and ovulated by the administration of FSH and LH according to the method of Foster and Hisaw. The process of ovulation and of subsequent luteinization was delayed significantly in the animals

which had been adrenalectomized prior to injection with FSH and LH. This was determined by histological examination of the recovered ova, by observation of the spontaneous motility of the uterus and of its reaction to adrenalin, and by measurements of the height of the uterine glandular epithelium and of the diameter of the uterine glands. Corpus luteum cysts were found in the ovaries of both normal and adrenalectomized animals, but were much more striking in size in the latter group. These huge lutein cysts, measuring 5 to 7 mm. in diameter, were present in the ovaries of cats showing adrenal insufficiency as well as in those adequately supported by cortin.—Authors' Summary.

GREENHILL, J. P. AND S. C. FREED. Virilism in women caused by androgenic therapy for menstrual disturbances. *J.A.M.A.* 112: 1573. 1939.

Two cases are reported in which testosterone was given to women in the treatment of menstrual disturbances. Attention is called to certain undesirable results. Growth of hair on the face and body as well as deepening of the pitch of the voice occurred in both cases and enlargement of the clitoris in one. Other disagreeable reactions were acne and increase in weight. While the desired clinical effects were secured the authors question whether these were worth the undesirable reactions. They advise that androgen therapy in women should be used only with caution and with the danger in mind of inducing virilism.—R. G. H.

HANSEN, E. H. Andrin (male hormone) in the human urine of male castrates. *Endokrinol.* 21: 9. 1938.

The urine of 12 castrated men showed the presence of considerable amounts of androgenic substance when tested on capons and on castrated and immature rats.—W. J. A.

HAROLD, L., AND G. EFFKEMANN, Absence of estrin induced luteinisation in female rats following section of the pituitary stalk (Fehlen der indirekten reaktiven Luteinisierung der Ovarien nach Follikelhormonzufuhr bei hypophysenstieldurchtrennten weiblichen Ratten). *Klin. Wchnschr.* 17: 940. 1938.

In infantile rats with pituitary stalk severed the daily injection of 100 R.U. of estrin for 3 days was not followed by the appearance of corpora lutea in the ovaries, although control animals similarly treated showed the expected luteinisation. The author concludes that a nervous connection with the hypothalamus is essential for the reaction. Further details to be published elsewhere.—M. H. F.

HARTMAN, C. G. The use of gonadotropic hormones in the adult rhesus monkey. *Bull. Johns Hopkins Hosp.* 63: 351. 1938.

Gonadotropic extracts derived from pregnant mare serum, pregnancy urine, menopausal urine and pituitary glands were injected into adult monkeys in an attempt to induce fertility during non-ovulatory menstrual cycles. Of 104 experiments, 32 resulted in a failure of any ovarian response. In 16 animals overstimulation resulted with undue hypertrophy of the granulosa and injury of the follicles. These injured ovaries subsequently recovered their normal function. Even where ovulation occurred the secondary changes in the uterus necessary for nidation were frequently unfavorable to the implantation of a fertilized ovum. The cycle was generally shortened to an average of 22 days by the injections. In only 7 cases could ovulation be attributed to the injections. The failures of the method are attributed to the difficulty of estimating correct dosage.—A. G.

HASSELMANN-KAHLERT, M. Pregnancy reaction in urine according to Hasselmann-Kahlert, modified Cuboni reaction (Schwangerschaftsreaktion im Urin nach Hasselmann-Kahlert, modifizierte Cuboni-Reaktion). *Zentrlbl. f. Gynäk.* 63: 447. 1939.

The reaction appears not to depend on prolan, and may be positive without pregnancy, even in males. If negative it is held to be conclusive in eliminating pregnancy. The test is a color reaction of empirical nature, differing from the Cuboni test for pregnancy in the mare, although the technic is the same.—E. L. S.

HERRELL, W. E. Studies on the endometrium in association with the normal menstrual cycle, with ovarian dysfunctions and cancer of the uterus. *Am. J. Obst. & Gynec.* 37: 559. 1939.

Employing the conventional methods for classifying endometria, the author finds cancer occurring only in uteri with proliferative or estrin types of endometria. Cancer is not seen if castration has preceded. The presence of ovarian cystic follicles in cases with cancer after the climacteric and of the finding of estrin in the urine in some of these cases all points to the importance of the persistent or uninterrupted action of estrogenic hormone as a factor in carcinogenesis.—E. L. S.

HERTZ, R., AND R. K. MEYER. The effect of surgical reduction of the amount of reacting tissue upon the quantitative effectiveness of testosterone propionate and estrone. *Am. J. Physiol.* 124: 259. 1938.

A part of the secondary sex organ tissue was removed from nearly half of 76 gonadectomized male rats and 37 ovariectomized female rats and the effectiveness of testosterone propionate and estrone in

causing growth of remainder of the secondary sex organ tissue determined. It was found that removal of a part of the seminal vesicles and/or a part of the prostate did not alter the response of the remaining tissue to testosterone propionate. Likewise the removal of one uterine horn did not alter the response of the remaining horn to estrone. The authors therefore conclude that the hormone concentration in the blood stream is of more significance in eliciting a response than is the ratio of hormone dosage to the reactive tissue.—S. L. Cohen.

Hinglais, H., and M. Hinglais. Sex of the fetus and humoral content of the mother's blood (Sex du foetus et titre hormonal du sang maternel). *Compt. rend. Soc. de biol.* 126: 582. 1937.

The blood of 27 pregnant women was assayed on rabbits for its gonadotropic content. In each case one test was made in the first part and one in the second part of pregnancy. There was no significant difference either in average content or individual variation between the blood of the 12 women who gave birth to boys and that of the 15 who gave birth to girls.—J. C. D.

Hirschmann, H., and O. Wintersteiner. Isoequilin A. *J. Biol. Chem.* 126: 737. 1938.

Equilin was converted by treatment with acid into a double-bond isomer, isoequilin A, to which the structure of a 14-epi-Δ_{8-9}-equilin was assigned. The new compound yielded on catalytic dehydrogenation a stereoisomer of equilenin, 14-epi-equilenin, which differed from equilenin by the configuration of carbon atom 14. Isoequilin A reacted with osmium textroxide to form hydroxequilin, probably 14-epi-Δ_{9-11}-8-hydroxyequilin. Spectroscopic measurements indicated that the fourth double bond in isoequilin A does not occupy the same position as that in the diol recently isolated from the urine of pregnant mares. It possesses about one-fifth of the estrogenic potency of estrone.—J. M. L.

Hirtz, G. The uterus from an endocrine point of view (L'uterus aus point de vue endocrinien). *Rev. franc. de gynec. et d'obst.* 33: 860. 1938.

An alcoholic extract of the uterus (myometrium and endometrium) was prepared from a non-gravid cow. By fractional extraction 1 cc. of the extract was equivalent to 1 gm. of fresh tissue. The extract was negative for estrogens and gonadotropic hormones. The active principles of the extract were not determined. By experiment with 18 rabbits, it was possible to substitute for the uterus in hysterectomized rabbits so that ovulation and formation of corpus luteum followed copulation. Clinical observations of many authors are cited, but no detailed clinical experiments of their own are reported.—J. P. P.

Kirsch, R. E. A study of the control of length of gestation in the rat with notes on maintenance and termination of gestation. *Am. J. Physiol.* 122: 86. 1938.

The placentae were retained until the date of expected parturition in all of 10 pregnant rats from which the embryos were removed between the 14th and 21st days of gestation. This was also true for 5 animals in which the embryos of only 1 uterine horn were removed. Paraffin pellets replacing the embryos or embryos plus placentae were likewise retained in utero until full term providing a few functional placentae were present in the same or contralateral horn (15 animals); but were aborted within 48 hours where no placentae were present (5 animals). The author also confirmed the findings of other workers that ovariectomy does not cause immediate (within 48 hours) expulsion of the placentae from rats in which the embryos have also been removed (15 animals) and that with special precautions one fetus can also be maintained. The author therefore concludes that the presence of the placenta is the most essential factor in maintaining the uterine quiescence characteristic of pregnancy; he considers the fetus to be unessential and the ovaries to play but a subordinate rôle in regulating the length of pregnancy. This function of the 'placento-uterine complex' is believed to be through the elaboration of progesterone or a similarily acting principle.—S. L. Cohen.

Holtz, F., and Rossman, E. Concerning the relation of the sex hormone to calcium metabolism and to the parathyroid glands (Über Beziehungen der Sexualhormone zum Kalkstoffwechsel und zu den Nebenschilddrüsen). *Ztschr. f. Geburtsch. u. Gynäk.* 116: 199. 1938.

In parathyroidectomized female dogs the serum calcium was maintained at a normal level by the administration of dihydrotachyesterin except when the dogs were in rut. Estrogen corrected hypercalcemia in a parathyroidectomized female dog. In a castrated, parathyroidectomized female dog, the lowered level of the serum Ca resulting from the administration of estrogen persisted in spite of the continued use of dihydrotachysterin. Parathyroidectomized dogs treated with progesteron and with androsteron did not show any consistent change in the serum Ca level. A patient with post-operative tetany is reported, in whom Roentgen castration was followed by a marked diminution in the need for dihydrotachysterin for the maintenance of the serum Ca. The authors conclude that estrogen increased the need for parathyroid hormone more than the male sex hormone and that this explains why tetany is more frequent in women than in men.—Milo Ritter White.

INK, J. Friedrich's reaction in pregnancy diagnosis (La reacción de Friedrich en el diagnóstico del embarazo). *Semana méd.* 45: 712. 1938.

Friedrich's reaction was investigated with a slight modification. Five cc. of urine was heated with 1 cc. of hydrochloric acid for 30 minutes. The formation of a dark flocculent precipitate in a brownish-red liquid indicates a positive reaction. Correct results were obtained during the first two months of pregnancy in 96.96% of the case, in up to 4½ months 98.63% and to 9 months 99.08%. The test remained positive after delivery for 20 days in all cases. It was also positive in carcinoma of the uterus. In men and non-pregnant women negative results were registered in 99.1% of the tests. The reaction does not depend on the presence of gonadotropic, follicular or corpus luteum hormones. Over 400 cases were tested.—A. E. M.

KAPELLER-ADLER, R., AND G. BOXER. On the influence of gonadotropic hormones on the destruction of histidine in the liver (Ueber den Einfluss gonadotroper Hormone auf den Histidinabbau in der Leber). *Biochem. Ztschr.* 293: 207. 1937.

The authors found that prolan and other gonadotropic hormones strongly inhibit the histidase of the liver. Twenty livers of human beings which had died of several diseases were used for the tests. Extracts of pregnancy urine had the same effect but not extracts from non-pregnancy urine. After inactivation by various procedures prolan preparations had no effect on liver histidase. The inhibitory action of the gonadotropic hormones on histidase, explains the presence of histidine in pregnancy urine. In one case of Cushing's syndrome histidine was also found in the urine and urine extracts inhibited the histidase.—E. Bozler.

KAUFMANN, C., AND E. STEINKAMM. The effect of unphysiological amounts of gonadal hormone on the genitals of castrated white rats (Über die Wirkung unphysiolgischer Mengen Keimdrüsenhormon auf das Genitale weiblicher kastrierter Ratten). *Arch. f. Gynäk.* 165: 358. 1938.

The authors studied the effect on the genital tract of castrated female rats of *a*) moderate overdosage (ca. 2085 to 2902 γ in 417 to 579 days) of estron, *b*), large overdosage (ca. 8600 to 25,920 γ in 140 to 452 days) of estron, *c*), simultaneous moderate overdosage (5.4 to 11.5 mg. in 63 to 135 days) of estradiol benzoate with marked overdosage (27 to 115 mg.) of progesterone, and *d*), overdosage of progesterone alone (27 to 309 mg. in 61 to 363 days). They found with *a*), 'prosoplastische' metamorphosis of undifferentiated cells into stratified epithelium in uterine horns and inflammatory foci of the entire genital tract, with *b*). the same changes as with *a*) plus fibrous changes in the submucosa, with *c*), the same pathological changes as with *b*), and with d), no pathological changes at all. In no instance were tumors produced.—F. A.

KIMURA, G., AND W. S. CORNWELL. The progestin content of the corpus luteum of the sow (*Sus scrofa*) during successive stages of the estrous cycle and pregnancy. *Am. J. Physiol.* 123: 471. 1938.

Sow ovaries, collected from the abattoir, were divided into 10 lots according to the various stages of the cycle and of pregnancy which they represented. For the determination of these stages microscopic examinations of the ovaries, uteri and/or fetuses were made. The corpora lutea were then removed, the different lots extracted and biologically assayed for progestin. During the first 3 days after ovulation the corpora lutea were found to contain appreciable amounts of progestin (16 rabbit units/kg.). This amount increased rapidly durnig the estrous cycle to approximately 33 RB.U./kg. on the 15th day, when retrogression sets in and after which progestin could not be detected in the corpora lutea. During pregnancy there was found a further sharp rise until the 20th day, and the progestin content remained high (50–65 RB.U./kg.) up to about the 100th day. At about this time a drop occurred and during the last 10 days (110th to 120th day) progestin was not found in the corpora lutea.—S. L. Cohen.

KLAFTEN, E. Concerning the structure and function through which the yellow body is produced by follicular hormone (Ueber Bau und Funktion der durch Follikelhormon erzeugten Gelbkorper). *Ztschr. f. Geburtsch u. Gynäk.* 115: 64. 1937.

Injections of from 50,000 to 200,000 I.B.E. of follicular hormone into isolated rabbits weighing over 1800 gm. produced luteinization. Exceedingly large doses over a long period of time produced metaplasia of the endometrium, edema, hemorrhage and necrosis of the uterine wall. In response to pituitrin, the induced corpora lutea behaved like corpora following ovulation. The appearance of the endometrium was not found to be a reliable test for luteal activity. The luteinizing effect was not demonstrated in infantile and juvenile rabbits, Doses required to produce necrosis were much greater than human therapeutic doses.—Milo Ritter White.

KOCH. F. C. Chemistry and biology of male sex hormones. *Bull. New York Acad. Med.* 14: 655. 1938.

Koch points out that at present 6 androgens have been found in nature and that 4 of them have been isolated from human urine. Only androsterone and dehydro-androsterone have been separated from

the urine of normal men. Whether all the androgenic activity in these urines resides in these compounds or whether others contribute remains to be determined. Thus far, pure androgen has not been separated from the urine of normal non-pregnant women. However, in the pregnant woman epiallopregnanolone-3, 20 has been separated from the urine as one of the androgens. In pathologic urines there undoubtedly are varying amounts of andrenosterone and pregnanetriol-3, 17, 20 in addition to androsterone, dehydroandrosterone and many other possible compounds of androgenic character. The normal type of androgen may be increased in concentration in pathologic conditions. In view of the possibilities of complex mixtures existing in various urines in various proportions it is probable that future advances in the quantitative studies on urine will depend to a large extent on the ability to differentiate quantitatively between the different androgens. However, for the present one must be content with the estimation of the total androgenic activity in tissue, body fluids and excretions in attempts to correlate the quantitative information with various endocrine disturbances possibly involving the gonads.—From abstract in J. A. M. A.

KORENCHEVSKY, V., AND K. HALL. Prolonged injections of male sex hormones into normal and senile male rats. *Brit. M. J.* 1: 4. 1939.

The authors undertook the investigation of the effects of prolonged treatment with male hormones on normal, non-castrated senile rats. The pure hormones, androsterone, transdehydroandrosterone, testosterone, and testosterone propionate were dissolved in sesame oil, of which 0.2 cc. was injected 5 times weekly. The sexual organs, hypophysis, adrenals, thyroid, liver and kidneys were weighed, fixed in Bouin's fluid and examined histologically, while the thymus, spleen and heart were weighed only. Of 63 rats, 20 senile and 31 adult males were injected for a period of 90 to 118 days. Of 12 senile rats, 6 were injected with oil, and 6 injected daily with 0.9 mg. androsterone for 24 days only. The changes observed in adult and senile rats, after the injection of the above hormones on the weight and histologic structure of the organs were similar. All the hormones with the exception of testosterone propionate depressed to a varying degree the development of the testis and the secondary sex organs. Testosterone stimulated the secondary structures. The mechanism is ascribed to suppression of the natural secretion of the testis hormones following depression of the hypophysis with consequent atrophy of the secondary sex organs. This atrophying effect is counterbalanced by direct stimulation of the secondary sex organs by the injected hormones, replacing completely or less completely the testicular secretion. The size and weight of the hypophysis was not changed except in 2 injected senile rats which had developed hypophyseal adenomata. It was noted that the male hormones do not prevent the development nor cause the regression of hypophyseal tumors. Some other changes noted were increased evolution of the thymus, decrease in weight of the liver, possible stimulation of the kidneys, some lipoid depletion in the cortex of the adrenals, nervous excitability, decreased fat deposition, and decreased gain in weight. There was no improvement in the senile appearance, and behavior of old rats: any rejuvenating effects of sex hormones were completely absent.—L. B. S.

KOTZ, J., AND E. PARKER. Endometrial patterns in menorrhagia and metrorrhagia. *Med. Annals, D.C.* 7: 371. 1938.

Fifty cases of menorrhagia and metrorrhagia were studied by means of the endometrial biopsy. The specimens were taken 1 to 3 days premenstrually in cyclic cases and repeatedly at weekly intervals in the acyclic cases. Tests for the estrogenic and gonadotropic substances were taken at the time of the biopsy. Functional bleeding and endometrial follicular hyperplasia have usually been considered to occur simultaneously. In 50 cases here reported, in which bleeding was either too frequent or too profuse, it was found that these endometria exhibited all degrees of differentiation as well as abnormalities manifested by hyperplasia, hypoplasia, persistent or delayed reactions in either the follicular or luteal phase of the cycle. Of the 50 cases reported, 27 or 54% of the endometria were in the secretory phase, 6 of which were associated with evidence of definite prolonged estrin action, while others showed abnormalities in which the possibility of hyperestrinism must be considered. In 23 cases or 46% the endometrium was of the follicular type, in 14 of which hyperplastic changes were evident, indicating either a relative or actual hyperestrinism.—Authors' Abstract.

KOTZ, J., AND E. PARKER. Endometrial patterns in dysmenorrhea. *Am. J. Obst. & Gynec.* 37: 116. 1939.

The occurrence of high levels of estrogenic substance in both the blood and urine has been noted in cases of functional dysmenorrhea. The purpose of this investigation was to determine if a deficiency of progestin might also be a factor. The endometrial biopsy affords the best method at present of estimating corpus luteum function. The 50 cases selected for study were those whose main but not necessarily only complaint was dysmenorrhea and in whom there was no organic systemic or pelvic pathology. From the study of the premenstrual endometrium it was concluded that of these cases, 18 or 36% had a normal endocrine function; 32 or 64% had some degree of ovarian failure. Of the failures, 30 cases or 60% were primarily corpus luteum deficiencies and 20 cases or 40% showed associated excessive follicular activity.

Only 2 cases or 4% showed deficiency of both hormones. Of the cases with normal hormone balance, 10 or 20% had pelvic congestion present as a possible etiologic factor. In 8 cases or 16% there was nothing to account for the dysmenorrhea.—Authors' Abstract.

LACASSAGNE, A., AND A. RAYNAUD. Injections of testosterone directly into the seminal vesicle of castrated rats to increase the sensivity of the biologic test for male hormone (Injection de testostérone dans la vésicule séminale du rat castré, pour accroître la sensibilité de ce test biologique de l'hormone male). *Compt. rend. Soc. de biol.* 126: 576. 1937.

Twenty days after castration the seminal vesicles of 3 to 4-month-old rats were exposed by abdominal incision. The outlet was gently constricted on one side by a ligature and 1/40 cc. of the solution to be tested introduced into the cavity. In 24 hours a dose (0.25 mg. of testosterone) which gave no detectable effect when injected simultaneously, produced clear cut results as judged by the height of the vesicular epithelium.—J. C. D.

LACASSAGNE, A., AND A. RAYNAUD. Results of studies on the reaction of the epithelium of the seminal vesicle to testosterone when injected into the gland cavity (Resultats obtenus dans l'étude de la reaction de l'epithelium de la vesicule seminale a la testosterone, par l'injection de l'hormone dans la lumiere de la glande). *Compt. rend. Soc. de biol.* 126: 579. 1937.

Testosterone acetate was injected directly into the cavity of the seminal vesicle in 50 rats 20 days after castration. The effect was measured in terms of the height of the epithelium. A dose of 0.0025 mg. gave a detectable reaction and one of 0.01 mg. a marked stimulation. The results, however, were not quantitative. Injections of estrone and estradiol gave very feeble reactions and those of progesterone, prolan, and AP extract gave none.—J. C. D.

LACKNER, J. E., H. WACHTEL AND S. SOSKIN. Unpredictability of the phenomena accompanying the menstrual cycle in normal women. *Am. J. Obst. & Gynec.* 36: 612. 1938.

In 15 normal women 60 kymographic records of uterine motility were obtained, half in the first half of the menstrual cycle. After the records had been finished endometrial biopsies were taken. During the recording the uterus was tested for spontaneous motility, sensitivity to posterior pituitary or adrenal medulla extracts. Urinary estrin and gonadotropic material were estimated. The findings were of infrequent spontaneous motility, 8 in 30 trials in the first half of the cycle. Responses to posterior pituitary extracts were less frequent than reported by Knaus in the first half of the cycle, more frequent in the last half, and again less frequent just before the flow. The effectiveness of pituitrin was greater than of pitocin, and pitressin was found definitely effective. No regularity was found for the excretion of estrin or gonadotropic hormones. The authors therefore cast doubt on the predictability of all such phenomena in normal cycles.—E. L. S.

LANGWORTHY, O. R., AND C. B. BRACK. The effect of pregnancy and the corpus luteum upon vesical muscle. *Am. J. Obst. & Gynec.* 37: 121. 1939.

The authors studied the vesical capacities of one series of rabbits before and during pregnancy and of another series following ovulation. There was a marked increase in vesical capacity during and immediately following the termination of pregnancy after which the vesical capacity gradually returned to normal limits. There was a similar increase in capacity in 50% of the cases following ovulation induced by the intravenous administration of antuitrin-S. The increase did not occur in a control series of castrated rabbits similarly treated with the same doses of antuitrin-S. The authors assume that the increased vesical volume is due to decreased tone in the vesical muscle and that this change in muscle tone is dependent on the hormones elaborated by the corpus luteum.

LAROCHE, G., H. SIMONNET AND E. BOMPARD. Influence of progesterone on the elimination of gonadotropic hormones in the urine (Influence de la progestérone sur l'élimination urinaire des principes gonadotropes). *Compt. rend. Soc. de Biol.* 126: 1159. 1937.

In 12 cases of castration and 2 of menopause, progesterone given subcutaneously was effective in reducing the output of gonadotropic hormone in the urine and relieving symptoms. A slight decrease in the hormone elimination was associated with a marked clinical improvement.—J. C. D.

LEBLOND, C. Effects of gonad stimulating factors A and B from the hypophysis on male and female gonads in pigeons (Action des gonadostimulines hypophysaires A et B sur les glandes genitales des pigeons males et femelles). *Compt. rend. Soc. de Biol.* 127: 1248. 1938.

Changes were followed in 6 male and 7 female pigeons. They received either FSH or LH from extracts of cattle hypophysis. The former produced enlargement of the testis or ovary and secondary development of the oviduct in the females. The LH produced a reduction in the size of the testis or ovary. It also stimulated the crop gland.—J. C. D.

LEIBOFF, S. L., AND A. B. TAMIS. A rapid and simplified method of extracting urinary estrogen. *J. Lab. and Clin. Med.* 24: 178. 1938.

The apparatus is designed for urine which has been hydrolyzed by acid, and chloroform is used as the sterol solvent. Two hours are allotted for extraction. This apparatus is now commercially available.—F. A.

LEGRAND, G. Investigations on fixation of gonadotropic hormones in serum of normal pregnant women and in those with hyperemesis. *Bruxelles-méd.* 19: 84. 1938.

Legrand demonstrated that in the course of normal pregnancy the gonadotropic hormone is absorbed by the proteins of the maternal blood serum. However, in women with hyperemesis gravidarium there exists, in addition to this absorbed hormone, a large quantity of hormone which is not absorbed by the serum albumins but is free. The presence of this nonabsorbed hormone seems to be related to the development of the symptoms of hyperemesis gravidarium. The author reports the clinical histories of two women with hyperemesis gravidarium. The serum of these women contained large quantities of free gonadotropic hormone.—Abstract from *J.A.M.A.*

LOESER, A. A. Therapeutic trails of diethylstilboestrol. *Brit. M. J.* 1: 13. 1939.

Patients with minor degrees of ovarian deficiency (4 cases of climacteric disorders, 4 with dysmenorrhea, 1 case of hypooligomenorrhea, 1 case of ovarian insufficiency following supravaginal amputation of the uterus, 1 case of pruritis vulvae) were treated with the synthetic hormone stilboestrol. The substance showed marked estrogenic effects proving itself a good substitute for the natural estrogens in the above forms of ovarian deficiency. In 6 of 15 cases, unpleasant secondary effects of nausea and vomiting were produced.—L. B. S.

LORENZ, F. W., I. L. CHAIKOFF AND C. ENTENMAN. The endocrine control of lipid metabolism in the bird. II. The effects of estrin on the blood lipids of the immature domestic fowl. *J. Biol. Chem.* 126: 763. 1938.

The injection of 500 to 3,000 R.U. of estrogenic concentrate from pregnant-mare urine produced a rise of whole blood lipids in 27 immature female fowl and 6 male birds of the same age. The effect was obtained within 12 hours after a single injection of 3,000 U but was more pronounced after 24 or 48 hours. All the lipid constituents, namely cholesterol, phospholipid and neutral fat, shared in the rise but the most pronounced effect was upon neutral fat.—L. O. Randall.

MACBRYDE, C. M. The production of breast growth in the human female by the local application of estrogenic ointment. *J.A.M.A.* 112: 1045. 1939. Percutaneous absorption of estrogenic substances by the human female, with especial reference to growth of the breast induced by local application of estrogenic ointments. *Proc. Central Soc. Clin. Research, J.A.M.A.* 112: 1104. 1939.

Several workers have produced local and general effects on the animal body by application of estrogenic substances to external surfaces. The application of appropriate amounts of the substances to any part of the skin has result in estrous changes and in mammary and uterine growth in certain spayed animals. DeFremery of Holland has reported the the best production of mammary growth in the virgin goat is accomplished by the application of estrogen directly to the skin of the udder. There are few studies of the effects of percutaneously applied estrogen in the human female, and there are no reports of the effects of estrogen directly applied to the breast to induce mammary development. Studies were performed on 3 women lacking mammary development and exhibiting signs of hypogonadism. Estrogenic ointments were found to induce unilateral mammary growth when only one side received applications of the active material and the other side was treated with the inactive ointment base. Vaginal smears changed from inactive to the active estrous state, and characteristic changes were noted in the result of the percutaneously absorbed estrogen.—Abstract from *J.A.M.A.*

MCCULLAGH, D. R., AND W. O. OSBORN. The male sex hormones of human urine and blood. *J. Biol. Chem.* 126: 299. 1938.

The androgenic material in normal human urine is all present in the inactive form and can be converted to the active form by acid hydrolysis, to yield about 50 I.U. per liter. The inactive material is soluble in butyl alcohol, less soluble in ethyl acetate and insoluble in benzene, chloroform and dibutyl ether. It is adsorbed by charcoal. The androgens in 30 cc. of blood were activated by boiling with sulphuric acid and extracted twice with 150 cc. portions of dibutyl ether. About 4 I.U. of androgenic material were found in 100 cc. of blood. This is all present in the inactive form.—J. M. L.

MCCULLAGH, E. P. Treatment of testicular deficiency with testosterone propionate. *J.A.M.A.* 112: 1037 1939.

Capon assays, using benzene extraction, in 20 normal men gave androgen outputs ranging from 16. to 86 international units (mean, 40.5). A simpler technic using dibutyl ether gave a mean of 35.1 I.U.

In 22 subjects of hypogonadism of various sorts, ranging in age from 14 to 64 years, the androgen output varied from 0 to 34 I.U. Clinical notes on several treated subjects are reported. It appeared that in high grade pre-puberal hypogonadism testosterone propionate gave symptomatic and anatomic changes in approximately the following order. Penile erections occurred promptly and there was an increase in the pubic and axillary hair. The penis grew rather markedly and the scrotum less so, and the prostate growth appeared to lag perceptibly in proportion. There was seen no consistent evidence of increased testicular size, although the testes appeared to be larger after treatment in one case. Nocturnal emissions occurred and the quantity of semen increased. No diminution in sperm count or inhibition of sperm production has been obvious where sperms were present. The larynx grew and the voice became lower. Facial acne appeared and the beard grew. Epiphysial closure has not exceeded its expected normal rate in cases in which testosterone propionate alone was used and in one case has not increased in 4 years in spite of marked advance in puberty. No constant change in basal metabolism has been observed. In cases of functional hypogonadism in the adult this treatment has been followed by complete relief of nervous and sexual symptoms. In castrates, nervous and vasomotor symptoms and impotence could be abolished by sufficient doses.—R. G. H.

HYPOPHYSIS

ADAIR, F. L., AND J. A. HAUGEN. A study of suspended human uterine muscle strips in vitro. *Am. J Obst & Gynec.* 37: 753. 1939.

Muscle strips were suspended in oxygenated Ringer's solution, and kymographic records made of contraction in response to pituitrin, pitressin, pitocin, adrenalin, and ergonovine. The ergonovine showed little activity save at term. Adrenalin caused reaction in nearly all muscles. The posterior lobe materials caused response at or near term, with greater result from pitressin than from pitocin. In the nongravid uterus the reactivity to all pituitary materials seemed less in uteri which had secretory endometria (corpus luteum effect).—E. L. S.

ALLENDE, I. L. C. DE. Effect of the hypophysis on the oviduct of batrachians (Influencia directa de la hipófisis sobre el oviducto de los batracios). *Rev. Soc. Argent. de Biol.* 14: 505. 1938.

In *Bufo arenarum* implantation of the glandular lobe of the pituitary from the same species produced a secretory phase in the glands of the oviduct in normal and ovariectomized animals. The oviduct is considered to correspond anatomically and functionally to the mammalian uterus. Mammalian anterior pituitary gave the same results in some experiments, in others it was not active. Estrone, testosterone propionate, and the gonadotropic hypophysoid substances of pregnancy urine did not modify the batrachian oviduct.—J. T. L.

ANGEVINE, D. M. Hypophysis and adrenals in anencephaly. *Arch. Path.* 26: 507. 1938.

A study of 20 anencephalic monsters is described. There were twice as many females as males in the series. Because it was usually impossible to recognize the hypophysis as a gross anatomic structure, a block of tissue was removed from the base of the skull, decalcified, and carefully sectioned. The anterior lobe of the gland was found in every monster, a pars intermedia was recognized in 6 and a pars nervosa in 5 instances. The adrenals were found in all the 18 monsters examined. The glands were all of the adult type because prenatal atrophy had apparently occurred in the developing adrenal tissue. The atrophy of the adrenals in anencephaly is probably the result of an impairment of the development of the hypophysis induced by changes in the tissues at the site of the base of the brain. No conspicuous changes were observed in other glands of internal secretion. Other congenital anomalies were found in about one-half the monsters. In several monsters the brain site was covered with hemorrhagic tissue in which there was a large amount of choroid plexus. It is suggested that this may be significant in the production of hydramnion.—From abstract in *J. Nerv. & Ment. Dis.*

BAUMANN, E. J., AND N. METZGER. Iodine in pituitary and some other tissues. *J. Biol. Chem.* 127: 111. 1939.

Pituitary glands from 18 human cases which gave no history of receiving iodine before death yielded on analyses from 7 to 14γ of iodine per 100 gm. of tissue. Several cases in which iodine was used as medication gave values up to 660γ per 100 gm. of dry tissue. The amount of iodine in the pituitary gland was not greater than that in other tissues except the thyroid.—J. M. L.

BOYD, E. M., AND D. W. WHYTE. The effect of extract of the posterior hypophysis on the loss of water by frogs in a dry environment. *Am. J. Physiol.* 124: 759. 1938.

Leopard frogs when treated with pituitrin (0.2 I.U. per 10 gm. of frog) showed a decreased loss of water from the skin as compared with untreated controls. Air currents increased the rate of evaporation markedly. The rate of evaporation at low temperatures was greatly decreased. Darkness had no effect on the rate. Pituitrin injected into frogs in room temperature 4° C. completely inhibited the loss of water

for a period of 4 to 6 hours. "Pitressin and pitocin were equally effective in inhibiting loss of water and neither one as effective as pituitrin in this respect."—Lena A. Lewis

BUNDE, C. A., AND A. A. HELLBAUM. Some chemical and physiological properties of the gonadotropic antagonist. *Am. J. Physiol.* 125: 290. 1939.

Extracts of sheep pituitaries containing either unfractionated gonadotropic principles or luteinizing principle alone, together with some factor which antagonized the effects of these principles were subjected to boiling and different PH levels, and the resultant solutions tested in immature female rats. Subjecting the pituitary preparations to a high PH (14° C.) was found to destroy both the antagonist as well as the gonadotropic principles; but a low PH under the same conditions, or boiling of the extracts, destroyed all the gonadotropic activity without affecting the antagonist. The possibility of other anterior lobe hormones withstanding this latter treatment is discussed.—S. L. Cohen

CASTLEMAN, B., AND S. HERTZ. Pituitary fibrosis with myxedema. *Arch. Path* 27: 69. 1939.

Castleman and Hertz cite a case of pluriglandular insufficiency without cachexia, clinically believed to be a case of myxedema. Except for the absence of cachexia, all the postmortem observations (fibrosis of the anterior lobe of the pituitary and atrophy of the thyroid, parathyroid, adrenal, ovary and uterus) are characteristics of Simmonds' disease. Pathologic and experimental evidence is presented which establishes primary disease of the anterior lobe of the pituitary in the pathogenesis. It is important to recognize that chronic pituitary insufficiency may exist. Such a condition should be suspected in all cases of typical myxedema or in cases of myxedema in which earmarks of other endocrine deficiencies are present. A negative thyrotropic assay of blood or urine in the untreated state of myxedema should suggest a primary deficiency of the pituitary.—Abstract from *J.A.M.A.*

CHANG, H. C., K. F. CHIA, C. H. HSU AND R. K. S. LIM. A vagus-post-pituitary reflex. Pressor component. *Chinese J. Physiol.* 12: 309. 1937.

It was found that stimulation of the central vagus in dogs with the neck crushed and with only vascular connections between the head and the trunk gives rise to a pressor response in the trunk which may be prevented either by interrupting the stalk of the pituitary or by removing the pituitary gland. These results indicate that vagal stimulation induces a reflex secretion of the posterior pituitary principle which is responsible for the pressor response by direct action in the circulation and not through the stimulation of a sympathetic center in the hypothalamus. Evidence is also presented showing that augmentation by cocaine or eserine does not necessarily indicate the presence of adrenergic or cholinergic activity respectively.—Authors' Abstract.

FLAKS, J., I. HIMMEL AND A. ZOTNIK. Polycythemia produced by extracts of the anteroir pituitary lobe proves the existence of a hematopoietic hormone (La polyglobulie provoquée par les extraits de lobe antérieur d'hypophyse prouve l'existence d'une hormone hématopoiétique). *Presse méd.* 46: 1506. 1938.

An abnormally high erythrocyte count was produced in normal rats by feeding of fresh or acetone extracted anterior pituitaries. The same result could occasionally be obtained in hypophysectomized rats. The reticulocytosis was quite conspicuous. Acid extracts were strongly active; alkaline extracts were almost inert. A deproteinized extrct, free from growth, gonadotropic and thyrotropic hormone had still a strongly hematopoietic effect. Changes in the bone marrow as observed after hypophysectomy also supported the theory of a hematopietic hormone.—A. E. M.

FOSTVEDT, G. A. In vitro studies of melanophore principle activity of the pituitary gland. *J. Pharmacol. & Exper. Therap.* 66: 14. 1939.

Melanophore hormone, from the anterior lobe of whale, accelerates the tyrosine-tyrosinase oxidase system over 100% in 4 hours.—E. L.

FRASER, A. M. The effect of magnesium on the response of the uterus to posterior pituitary hormones. *J. Pharmacol. & Exper. Therap.* 66: 85. 1939.

Systematic studies have shown that postlobin-O has a greater oxytocic action in van Dyke-Hastings solution than in Locke's solution because of the magnesium salt present. Postlobin-V has its maximum effect on the uterus when the concentration of $MgCl_2$ is 50 mg. per 100 cc. When the concentration of $MgCl_2$ is 100 or 200 mg. per 100 cc. postlobin-V and postlobin-O have the same oxytocic activity Magnesium chloride decreases the uterine response to histamine and ergobasine tartrate.—E. L.

FRIEDMAN, M. H. AND G. S. FRIEDMAN. Gonadotropic extracts from green leaves. *Am. J. Physiol.* 125: 486. 1939.

The preparation of an extract from immature oat grass capable of producing ovulation upon injection into mature rabbits is described. Similar treatment of alfalfa leaf meal yielded extracts which were

not always potent; immature Sudan grass, timothy, barley and rye grass yielded only impotent extracts. The chemical properties of the active extracts, including its pronounced instability at low temperatures, were observed to simulate the properties of the gonadotropic material in AP extracts.—S. L. Cohen.

Fugo, N. W., and E. Witschi. Removal of the hypophyseal primordium in the chick during the second day of incubation. *Acta Biologica Latvica* 8: 73. 1938.

The primordium of the pituitary was removed from 429 embryos incubated for from 33 to 38 hours. Of these 123 developed to an age of 12 to 21 days. In the absence of the hypophysis the gonads and gonoducts developed and differentiated in a normal, or nearly normal manner up to and through the 19th day. The thyroids of the experimental embryos differentiated normally but were smaller and deficient in colloid as compared with controls of the same age.—R. G. H.

PANCREAS

Aitken, R. S. Duration of action of zinc protamine insulin. *Lancet* 235: 678. 1938.

The patients investigated were at bed rest and given weighed and measured diets (3-hour feedings) with an unrestricted fluid intake. No blood sugar determinations were made, but the urinary excretion of sugar in 3-hour periods was measured and the absence of acetone recorded. It was noted that zinc protamine insulin had a maximal effect on the carbohydrate metabolism without 3 to 6 hours of the injection, and the total duration of the effect of doses between 15 to 100 U is 15 to 60 hours.—L. B. S.

Baker, A. B. Cerebral lesions in hypoglycemia. Some possibilities of irrevocable damage from insulin shock. *Arch. Path.* 26: 765. 1938.

Two cases of hypoglycemia in patients 60 and 64 years old, respectively, are described in which extensive changes were observed within the vessels, myelin sheaths and glia. Hypoglycemic lesions are divided into (1) cerebral petechiae, varying from discrete extravasations to large hemorrhages; (2) large areas of encephalomalacia, demylinization, and cyst formation; (3) diffuse generalized glial proliferation and extensive gliosis around the areas of softening. These findings should caution one in the use of hypoglycemia as a therapeutic agent.—L. B. S.

Bay, A. P., Hypothermia in induced hyperinsulinism. *Illinois M. J.* 75: 369. 1939.

This is a record of the observations on the hypothermia associated with insulin hypoglycemia in man. The author infers that the resulting temperature fall in insulin shock therapy is due to physical heat loss plus another factor of chemical and endothermic nature.—I. B.

Bridge, E. M., and E. A. Winter. Diabetes, insulin action, and the respiratory quotient. *Bull. Johns Hopkins Hosp.* 64: 257. 1939.

Diabetic children were studied in an attempt to correlate the respiratory quotient to the general improvement and to blood-sugar variations after insulin treatment. There was no direct relationship observed between insulin action and the R.Q. Hypoglycemia from overdosage with insulin was not due to an abnormal rate of combustion of glucose, but probably to an excessive deposition of glycogen. The blood-sugar level by itself was not an adequate criterion for judging the regulation of diabetes.—A. G.

Cowin, W. C. Decreased resistance to hypoglycemia on successive days of administration of insulin. *Am. J. Physiol.* 125: 227. 1939.

When non-diabetic dogs were given insulin on succeeding days (1 U per kg. every 2 hours until convulsions occurred) convulsions occurred earlier on the second day and still earlier on the third day The resistance to insulin then became stabilized so that in all succeeding days there was little if any variation in response. In more than 110 experiments on 26 dogs this peculiar decrease in resistance oc curred invariably. No change in the rate of development of hypoglycemia, the depth of hypoglycemia, the tolerance to glucose, glycogen content of liver, inorganic phosphate or potassium of the serum could be found to explain the phenomenon.—From Author's Summary.

Hanzlik, P. J., W. C. Cutting and G. B. Robson. Agent promoting intestinal absorption of insulin. *J. Pharmacol. & Exper. Therap.* 66: 17. 1939.

Though certain agents (alcohols, dyes, local anesthetics, etc.), which increase permeability, promote hyperemia, and lower surface tension, increase the absorption of insulin from intestinal loops in animals, their action was either uncertain or negative in man.—E. L.

Himwich, H. E., Z. Hadidian, J. F. Fazekas and H. Hoagland. Cerebral metabolism and electrical activity during insulin hypoglycemia in man. *Am. J. Physiol.* 125: 578. 1939.

The electroencephalogram and oxygen utilization of the brain were observed simultaneously in 5 schizophrenics during insulin therapy. The frequency of the alpha rhythm and O_2 consumption declined

during hypoglycemia, whereas the delta index rose. These results show, partly in confirmation of previous work, the importance of carbohydrates for the metabolism of the brain and indicate a close relationship between brain metabolism and the electroencephalogram.—E. Bozler.

KASTEIN, G. W. Insulin intoxication. *Ztschr. f. d. ges. Neurol. u. Psychiat.* 163: 322. 1938.

A fatal case of insulin intoxication in a 32-year-old female patient suffering from a second episode of catatonic schizophrenia is presented. The course of insulin treatment had been beneficial. About a month previously, the patient had had a protracted shock lasting for several hours, but after a 5-day interval treatment had been resumed. The fatal coma set in after shock interruption, following a normal hypoglycemic coma. Nasal gavage awakened the patient at 11.45 A.M. so that she could drink independently, but an increasing motor agitation supervened, she began to shout and gesticulate, then went back into coma. She was again aroused with 10 gm. intravenous glucose, but after vomiting became comatose once more. Thereupon 10 gm. glucose were injected twice unavailingly. At 5.00 P.M. when blood sugar was still only 30 mg.%, 20 gm. glucose plus 0.2 gm. metrazol were injected with no result. The patient remained more or less deeply unconscious until death 20 days later. During this period glucose was administered intravenously or rectally in accordance with the blood sugar curve which showed an extraordinary lability during the first 3 days. After 2 insulin injections on the third day (15 to 20 U) it was possible to keep the blood sugar fixed at about 100 mg.% through rectal glucose administration. Regular measures employed were hypodermoclysis, metrazol, coramine and caffein, as well as transpulmin to ward off pulmonary complications. In protracted shock the author has noted the following neurological symptoms: increase of tonus in certain muscular groups with adoption of rigid attitudes in the limbs similar to those following section of the midbrain; rough, swinging movements of the arms and legs of a slow, vague, groping character which may continue for hours; quick, tic-like movements and spasms of certain muscle groups; regular backward movements of the eyes; great lability in pulse, respiration and blood pressure; epileptic attacks. Histologically, evidence points in this case to a general toxic metabolic process in all the ganglion cells plus local changes in ganglion and glia cells as the result of vascular changes. These include primary intimal cell growth, secondary thrombus, leucocyte thrombus and extravasation, hemorrhage through diapedesis, rhexis and vascular dilatation with stasis, and conspicuous capillary increase in the infarcts.—Abbreviated abstract, Science Library, Neuro Psychiatric Institute, Hartford Retreat.

PARATHYROIDS

ALBRIGHT, F., H. W. SULKOWITCH AND E. BLOOMBERG. A comparison of the effects of vitamin D, Dihydrotachysterol (A.T. 10), and parathyroid extract on the disordered metabolism of rickets. *J. Clin. Investigation* 18: 165. 1939.

Using the same 17-year-old patient with rickets resistant to ordinary doses of vitamin D the authors report further studies, which also amplify recent papers on the therapy of tetany. The results demonstrate that the A.T. 10 causes less increase in urinary phosphorus in rickets than in parathyroid deficiency, due to the increased absorption of Ca, which tends to increase serum Ca and thereby to inhibit parathyroid secretion and consequently P excretion. The A.T. 10 is less potent as an antirachitic than is vitamin D, and the parathyroid extract is not antirachitic; vitamin D has very little ability to stimulate P excretion.—E. L. S.

ASK-UPMARK, E. The parathryoids and the body temperature. *Acta Med. Scandinav.* 96: 481. 1938.

The behaviour of body temperatures in 4 cases of hyperparathyroidism was studied. Three of the cases showed an increased temperature which became normal in 2 instances after operation. In the 4th case the temperature was subnormal and approached normal values after removal of the adenoma. The temperatures in hyperthyroidism may present an undulation like that in the Pel-Ebstein phenomenon, and this was thought to be due to disturbances in the reticuloendothelial system, particularly the bone marrow. Parathyroid disorders are prone to have seasonal fluctuations.—J. M. L.

HIGHMAN, W. J., AND B. HAMILTON. Hyperparathyroidism secondary to experimental renal insufficiency. *Arch. Path.* 26: 1029. 1938.

Three dogs survived a renal insufficiency operation, which consisted of an approximate heminephrectomy at one operation, and a contralateral nephrectomy at a later operation. The dogs were fed a normal diet of milk, bread, meat and each was given a tablespoon of cod liver oil. Ca, P and N.P.N. were determined on blood samples. The experiments showed that the increased activity of the parathyroid glands were detectable within the first month of renal insufficiency with hyperplasia of the glands appearing approximately at the same time. It is the opinion of the authors that the parathyroid changes (functional and morphologic) are progressive, the extensiveness of the change being related to the time element involved.—L. B. S.

HURXTHAL, L. M. The treatment of tetany with dihydrotachysterol (A.T. 10). *The Lahey Clinic Bulletin* 1: 15. 1939.

Dihydrotachysterol is a derivative of irradiated ergosterin. It is reported as highly efficacious in the control of parathyroid tetany. It is apparently not anti-rachitic. Hurxthal reports briefly on 6 cases of chronic tetany successfully treated. In all cases the blood Ca was brought to normal and readily maintained at that level. The initial dosage varied from 3 to 15 cc. in some cases then, as symptoms came under control, was tapered down to 1 or 2 cc. daily. Ca. was used as an adjuvant; it materially reduced the requirement of A.T. 10. The caution is noted that in over-dosage hypercalcemia may be produced—a condition that is to be suspected when headache, nausea and general malaise intervene. After the dosage is stabilized weekly determinations of blood Ca suffice for therapeutic orientation.—R. G. H.

JOHNSON, J. W. Primary hyperparathyroidism with extensive renal calcification and secondary hyperplasia of the parathyroids. *Am. J. Path.* 15: 111. 1939.

A case of hyperparathyroidism is reported associated with an adenoma of the right lower parathyroid, accompanied by calcification of a horseshoe kidney as disclosed by x-ray. Removal of the parathyroid tumor was followed by termination of the hyperparathyroidism. There developed subsequently hypertension, cardiac hypertrophy, renal insufficiency and death in uremia. The important lesions disclosed at autopsy were extensive calcification of the renal pyramids of a horseshoe kidney, widespread arteriolar sclerosis and necrosis, arteriolar nephrosclerosis and hyperplasia of the three remaining parathyroid glands.—Author's Summary.

MARGOLIS, H. M., AND G. KRAUSE. Postoperative parathyroid tetany. *J.A.M.A.* 112: 1131. 1939.

A case is reported of postoperative parathyroid tetany, refractory to treatment and progressively more disabling, despite the use of large doses of Ca, halibut liver oil and viosterol and solution of parathyroid. The symptoms were promptly and entirely relieved and the metabolic effects of the parathyroid insufficiency satisfactorily controlled by means of dihydrotachysterol.—R. G. H.

THYROID

BARTELS, E. C. Serum-protein studies in hyperthyroidism. *New England J. Med.* 218: 289. 1938.

The serum protein content was determined by the micro-Kjeldahl method in 59 cases of hyperthyroidism. In 31 cases 3 examinations were made on admission, just before operation and after 3 months. In 28 other cases no post-operative examination was made. The hippuric acid excretion test of Quick was carried out on most of the cases. The average value for serum protein on admission was 6.4 gm. % and 63% of the values were below normal. Preoperative treatment resulted in some improvement as only 37% of cases were below normal at time of operation. The fall in blood proteins was more marked with respect to serum albumin which was below normal in 73% of the cases on admission, with an average value of 3.4 gm. %. The albumin-globulin ratio was also reduced. No direct relationship was found between protein content and B.M.R. or duration of hyperthyroidism. A rough correlation was found between total serum and hippuric acid excretion. Low values for protein were found in the more severe cases which required a 2-stage operation. After operation the protein values return to normal with an average value for total protein of 7.8, 4.8 for albumin, and 2.5 for globulin. The change in serum protein was attributed to disturbances of liver function. The edema of hyperthyroidism not due to cardiac or renal insufficiency may be caused by a fall in serum protein below the critical level.—J. M. L.

BARTELS, E. C., AND G. E. HAGGART. Osteoporosis in hyperthyroidism. *New England J. Med.* 219: 373. 1938.

A review of the literature of 5 cases with single or multiple spontaneous fractures on the basis of the decalcifying process in hyperthyroidism is given and details of two more cases are reported. Both patients had hyperthyroidism of long duration, 9 and 17 years, respectively, and both had suffered multiple fractures. In most cases the hyperthyroidism is of a mild type which is allowed to continue for many years before it is diagnosed and treated. One of the chief complaints is that of pain in the back, usually due to crushed vertebrae.—J. M. L.

BISGARD, J. D. Relation of hyperthyroidism to hypertension. *Arch. Int. Med.* 63: 497. 1939.

As a physiologic response to hyperthyroidism, the systolic blood pressure is usually slightly or moderately elevated. It recedes to the normal level with relief of hyperthyroidism. In a series of 265 cases studied by Bisgard, hyperthyroidism was associated with true essential hypertension in approximately 8% and with elevations of the systolic pressure above the physiologic level (above 150 mm. of mercury) in 30%. On the basis of changes of blood pressure following relief of hyperthyroidism, two types of cases are discernible: (1) cases with fixed or established essential hypertension, in which the blood pressure and the course of the vascular disease are not significantly influenced by relief of hyperthyroidism, and (2) cases with latent or labile essential hypertension, in which the blood pressure shows

considerable reduction after relief of hyperthyroidism or approaches a normal level and maintains this level for a long period. In some cases of the second type the pressure subsequently ascends. In these cases there is excessive response in blood pressure to exercise and to the cold pressor test of Hines and Brown. It is suggested that the relation of hyperthyroidism to hypertension in these cases is provocative, that hyperthyroidism merely precipitates or exaggerates hypertension which is latent. It is further suggested that in both types there is arteriolar disease, differing only in degree. In the first type the disease is more advanced, rendering the vascular bed inadequate for even a normal volume of blood flow. In the second type the disease may be designated as pretensive hypertension, which progresses to the condition of the first type with the advance of time.—I. B.

COHEN, R. A., AND R. W. GERARD. Hyperthyroidism and brain oxidations. *J. Cell. & Comp. Physiol.* 10: 223. 1937.

The objective of this study was the demonstration of the steps in the oxidation sequence which are hastened by the thyroid hormone and the cell enzymes activated by it. The brains of normal and those of hyperthyroid rats were compared for their ability to oxidize various substrates, and the relative effects of specific respiratory inhibitors were determined. The brain of a hyperthyroid animal had a distinctly increased respiratory rate. Thyroxinized brain burned substrates with oxygen at a rate 30% or more greater than normal. The catalytic activity, furthermore, was not increased in similar proportions for all oxidations, but was specific for certain substrates. Substances which occur in the sequence of carbohydrate oxidation or glycolysis were more rapidly oxidized by the brains of hyperthyroid than by those of normal animals. Analysis of the respiratory changes indicated that the absolute concentration of various enzyme systems is greater in the brains of hyperthyroid than in those of normal rats, and that certain dehydrogenase are increased relatively more than oxidases.—Abstract from *Arch. Neurol. & Psychiat.*

DRILL, V. A., AND C. R. SHERWOOD. The effect of vitamin B_1 and the vitamin B_2 complex on the weight, food intake, and estrual cycle of hyperthyroid rats. *Am. J. Physiol.* 124: 683. 1938.

Rats (250 to 300 gm. each) that had become hyperthyroid by feeding thyroid gland lost weight rapidly unless additional quantities of B vitamins were administered. Daily doses of 500γ crystalline vitamin B_1 checked the loss of weight, and the further addition of vitamin B_2 complex permitted regaining the lost weight. Female rats receiving 100 mg. of thyroid daily responded to vitamin supplementation equally as well as males receiving only 50 mg. of thyroid daily. Vitamin B_1 greatly stimulated the food intake of hyperthyroid rats but addition of vitamin B_2 complex was essential for weight recovery. Limited food intake, even with adeqaute amounts of vitamins, did not permit regaining lost weight. Vitamin treatment also enabled hyperthyroid rats that were entering diestrus to maintain normal estrous cycles.—H. W. Schultz.

GIBSON, J. G. 2D., AND A. W. HARRIS. Clinical studies of the blood volume. V. Hyperthyroidism and myxedema. *J. Clin. Investigtaion* 18: 59. 1939.

Using the Evans blue dye method 25 thyrotoxic and 7 myxedematous patients were studied. Blood volume tends to vary above normal in proportion to the basal rate, but the determination is not of especial value diagnostically because of the overlapping with normal values, as well as the technical difficulty of the method. Therapy which returns the basal rates toward normal restores blood volumes also. The volume is below normal in myxedema, also in proportion to the oxygen use.—E. L. S.

HANSSEN, P. Myxedema and ascites. *Acta Med. Scandinav. Supplement.* 90: 277. 1938.

The history is given of a woman, aged 59 years, who was subjected to 157 paracenteses and from whom 1357 liters of ascitic fluid were removed over a period of 8 years. At this time a thorough check-up revealed clinical symptoms of myxedema, enlarged heart and hypercholesterolemia. After 5 weeks' treatment with thyroidin the ascites and myxedema cleared up. Reference is also made to a similar case of Professor Bergmark of Uppsala that improved on thyroid treatment. The ascites is not due to heart disease, but must arise from the same cause as the subcutaneous edema of myxedema.—J. M. L.

HERMAN, H. A., W. R. GRAHAM, JR., AND C. W. TURNER. The effect of thyroid and thyroxine on milk secretion in dairy cattle. *Univ. Missouri. Col. Agriculture Exper. Sta., Research Bull.* 275, 1938.

Thyroxine was injected subcutaneously and desiccated thyroid given orally to 13 cows in various stages of lactation. Thyroid at 2 ounces daily given in the declining phase of lactation brought about an 18.2% increase in fluid and 35% increase in butter-fat yield. Cows at the peak of lactation or nearing the end of that period showed no increase in volume but a slight increase in fat. During the decline phase of lactation thyroxine in 5 mg. daily doses caused an 11% increase in volume and 22% in fat. Doubling the dosage raised the output to 23 and 37% respectively. The pulse rate was somewhat increased and there was slight loss of body weight. Injection of thyroxine 3 to 7 weeks post-partum caused

diminution in both milk and fat with marked increase in pulse and respiratory rates and rapid loss in weight. Ten milligrams of thyroxine was found to produce an effect approximately equal to 2 oz. of desiccated thyroid. The authors suggest that the relative inefficiency of the thyroid substance might be due to its inactivation by prolonged retention in the stomachs.—R. G. H.

McDonald, C. H., R. W. Boyle and A. J. DeGroat. Hyperthyroidism and cardiac glycogen. *Am. J. Physiol.* 124: 742. 1938.

Ten dogs were given daily 0.4 gm. thyroid substance per kg. of body weight and after several days the glycogen content of parts of the heart was determined. Unless tachycardia lasted for a considerable period the cardiac glycogen was unaffected. The authors believe that intravenous injections of epinephrine lower the glycogen content of the heart in these hyperthyroid animals. This reduction is said to occur even if insulin and glucose are given. Only minor pathological changes were noted in these hearts.—G. C. Ring.

Marine, D., and S. H. Rosen. Effects of cryptorchidism and castration on exophthalmos in rabbits and guinea pigs. *Am. J. Physiol.* 121: 620. 1938.

The effects of castration and cryptorchidism on the exophthalmos resulting from thyroidectomy in 8 rabbits and that naturally present in 19 guinea pigs were studied. Castration caused regression of the exophthalmos in the rabbits but had no effect on the eye condition in the guinea pigs. Cryptorchidism had no effect in either producing exophthalmos in normal rabbits nor of alleviating this condition, once established in the animals by thyroidectomy. Similar treatment in normal guinea pigs, however, caused a slight and transient exophthalmos. The results for the rabbits are interpreted as indicating that only the non-germinal tissue of the gonads plays a rôle in the sympathetic stimulations which cause exophthalmos after thyroidectomy. It is suggested that the differences observed for the rabbit and guinea pig may be due to a difference in partition of gonadal function between adrenal cortex and interstitial cells in the 2 animals.—S. L. Cohen.

Picardo, C., and W. Rotter. Modification of thyroid function by various immunity reactions (Über Modifikationen der Schiddrüsen funktion bei verschiedenen Immunitätsreaktionen). *Endokrinol.* 21: 93. 1938.

By injecting antithyroid serum and thyrotropic hormone into guinea pigs under various experimental conditions it was possible to produce modifications of the structure of the thyroid, sometimes indicating hyper-, at others hypo-function. It is supposed that in the production of these appearances a significant rôle is played by the immune reaction.—W. J. A.

Puppel, I. D., and G. M. Curtis. Iodine balance in exophthalmic goitre. *Arch. Path.* 26: 1093. 1938.

Three persons were studied as controls over a period of 39 days. Five patients with exophthalmic goiter were investigated over a total period of 186 days. The normal person maintained on a low intake of iodine averaging 87γ per 3-day period, over a period of 24 days showed continuous negative iodine balance of 126γ per 3-day period. The total excretion of iodine averaged 213γ per 3-day period. The greatest excretion was through the urine, 72%, feces 15%, sweat 13%. The normal blood iodine averaged 4.3γ per 100 cc. Only when iodine was furnished in excess of the amount excreted daily was there a positive iodine balance. The significance of this negative balance in normal persons with low intake of iodine is not clear. Three patients with exophthalmic goiter maintained on a low intake of iodine, showed a great increase in the excretion of iodine, particularly through the feces. This resulted in a negative iodine balance of 2 to 3 times the normal. The total excretion was 414γ per 3-day period, the greatest excretion through the urine, 49%, feces 40%, sweat 11%. Two patients with exophthalmic goiter were maintained on an intake of iodine sufficient to keep a normal person in positive iodine balance. However, these patients also showed a negative iodine balance.—L. B. S.

Puppel, I. D., K. P. Klassen and G. M. Curtis. Calcium balance in thyroid disease. *Proc. Central Soc. Clin. Research, J.A.M.A.* 112. 1104. 1939.

Three normal persons maintained on a constant low intake of Ca remained in negative Ca balance. Four patients with exophthalmic goiter similarly maintained revealed an increased mobilization of Ca and a tremendous increase in excretion of this element though both the urinary and the gastrointestinal tract. Likewise, two patients with toxic nodular goiter showed an increase in excretion of Ca but only slightly over normal. On the other hand, two patients with nontoxic nodular goiter remained in physiologic Ca balance. Increased feeding of Ca to a normal person on a low intake of Ca produced an immediate positive Ca balance. Similar increased feeding of Ca to a patient with hyperthyroidism resulted in a retention of Ca which was 6 times the normal and a consequent positive Ca balance. One patient with exophthalmic goiter had shown an increase in the negativity of the Ca balance prior to subtotal thyroidectomy. Examination 2 years postoperatively revealed an absence of all characteristic symptoms and a B.M.R. of −9%. Studies of Ca balance showed that the excretion of Ca was also normal and that the Ca balance had returned to within normal limits.—Abstract from *J.A.M.A.*